THE VETERINARY JOURNAL

EDITORS: T. DALLING, *M.A., M.R.C.V.S.* J. McCUNN, *M.R.C.V.S., L.R.C.P.*

Vol. 104 September, 1948 No. 9

EDITORIAL

NEWCASTLE DISEASE IN POULTRY

In the early part of 1947, Newcastle disease was diagnosed in this country and since then many outbreaks of the acute disease have been confirmed and dealt with under the Fowl Pest Order of 1936. Further Orders made later have concerned the movements and sales of live poultry. Recent reports indicate that the disease is being controlled to a marked extent, as shown by the reduction in the number of outbreaks and, with a continuation of the application of the measures now in force, we look forward to the day when Newcastle disease in its virulent form will cease to exist in Great Britain. The Ministry of Agriculture and Fisheries have, however, issued a statement on the presence in the country of a "low" form of the disease. It is stated that "there are at least two forms of Newcastle disease. One, the European form, is more virulent than the form now prevalent in the U.S.A. Until recently there was no reason to think that a low form of the disease existed in this country, but, as a result of the testing of some birds that had been entered in certain laying trials, it has been proved that a low form of Newcastle disease exists here." It was in connection with the enquiries made to determine the origin of infection in dealing with a virulent outbreak that examination of birds at this laying trial were carried out, although the birds at the laying trial had not, so far as is known, had any actual contact with diseased stock. The test referred to is the hæmoagglutination-inhibition (H.I.) test. The positive reactors in the laying trial had good health and egg-production records. Tests carried out at a later date on birds in contact with the positive reactors gave negative results: this seems to indicate that the reacting birds had recovered from the disease when they entered the trials and had not been responsible for transmitting infection to other poultry. Tests were also made on birds on the home premises of a number of poultry-keepers who had sent birds to the trials, and reactors were found there. It is also reported that reacting birds were found on some large hatcheries; on one hatchery "it was discovered that nearly three years ago some trouble had been experienced in young chicks (two to four weeks old), but that the only symptoms that those chicks had displayed were similar to those normally associated with slight colds. There was no unusual mortality among such chicks."

XUM

This low form of the disease in this country, although showing some similarity to the type which has been recognised in America for some years, seems to be less virulent in nature; in America the disease causes a serious decline in egg production, while in this country nothing of this has been found.

The incidence of the condition in this country is not known, nor do we know anything of "the likelihood of the infection assuming a more virulent character, but since this low form of the disease has existed, in all probability in this country for three years and, so far as is known, there is nothing to suggest that prior to the importation of the more virulent (European) form it has ever assumed a virulent character here, the experience in this country seems to be somewhat reassuring on this point." It is also stated that "though there is no data on which a positive statement could be made that this low form of disease exists in many districts, the Ministry can only conclude that it must be present on many premises."

So far as dealing with this type of infection is concerned, the statement says "the blood-testing for determining whether or not birds are or have been affected by Newcastle disease is a delicate one, occupying much time and calling for the services of highly trained staff: its application on a wide scale in the field would, therefore, be impracticable. For these reasons and because it could not be diagnosed clinically—frequently, birds affected with this low form show no signs of disease or ill-health—it is clearly impossible to deal with this form of the disease by the slaughter method. That method is suitable and is being pursued against the more virulent (European) form of the disease. Should, however, this low form of Newcastle disease assume a form which could be recognised clinically, it would be dealt with on the same lines as those followed in the case of the European form."

That is the present position: doubtless we will hear more of the disease in the future and whether any evidence is obtained of its assuming virulent characteristics, we have here a problem of importance for the research worker and the observer in the field, and by their joint effort we may look forward hopefully to an elucidation of some of the outstanding features, especially its easier diagnosis, whether it may pass to a virulent form and further possible methods of control.

THE ANNUAL N.V.M.A. CONGRESS

We draw our readers' attention to the Annual Congress of the N.V.M.A., to be held this year at Southport from September 8 to 15, inclusive. There is an excellent and interesting selection of subjects for discussion. We missed the annual congresses during the war period, although successful meetings on a reduced scale were held on several occasions. Congresses are the meeting places for scientific discussions and they are to be encouraged. The social side must not be forgotten, for it is often the only occasion when old friendships are renewed and new friends are acquired.

We wish the Congress every success.

L. Guy Anderson, D.V.M., M.R.C.V.S., President, N.V.M.A.

N.V.M.A. CONGRESS 1948

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PROGRAMME

The following is the programme of the Association's Congress, to be held in Southport from September 8 to 15 (inclusive). All meetings will take place in the Cambridge Hall, Lord Street, except where otherwise stated.

Wednesday, September 8

8.30 p.m. to 10 p.m. Reception by the Lancashire Division (Prince of Wales Hotel).

Thursday, September 9

10 a.m. Opening of Congress by the Mayor of Southport (Councillor E. W. Raynor, J.P.).

10.30 a.m. Opening of Exhibition of Drugs and Surgical Instruments, etc., by President, N.V.M.A. (Victoria Hall).

11 a.m. Paper: "The Tuberculin Test." By T. Dalling, M.A., M.R.C.V.S., F.R.S.E. Openers of discussion: G. H. Arthur, M.V.SC., M.R.C.V.S., and J. McWilliam, M.R.C.V.S. Chairman: J. Holroyd, F.R.C.V.S., D.V.S.M. Recording Secretary: A. R. Jennings, B.V.SC., M.R.C.V.S.

3 p.m. Public address: Sir John Boyd Orr, D.S.O., M.C., LL.D., F.R.S., "World Food and Agriculture." Chairman: Professor J. G. Wright, M.V.SC., F.R.C.V.S.

5 p.m. Meeting of Council (Town Hall).

8 p.m. Civic Reception (Floral Hall).

Friday, September 10

9.30 a.m. Coaches leave Southport for Liverpool University; welcome by Vice-Chancellor in the Arts Theatre, at 10.30 a.m., followed by tour of the buildings and demonstrations in various departments. Luncheon in the Union, and then to "Leahurst," University of Liverpool Veterinary Field Station, for further demonstrations and tea. Excursions for ladies to Liverpool and New Brighton or to Liverpool and Chester.

8.30 p.m. President's Reception (Prince of Wales Hotel).

Saturday, September 11

10 a.m. Annual General Meeting.

12.15 p.m. Congress Photograph (Floral Hall).

Sunday, September 12

9 a.m. Excursion to Keswick via Windermere, including sail round Derwentwater.

Monday, September 13

10 a.m. Paper: "Equine Accidents in the Fields of Sport." By Henry Sumner, M.R.C.V.S., D.V.H. Openers of discussion: E. Brayley Reynolds, O.B.E., M.R.C.V.S., and J. S. M. Cosgrove, M.R.C.V.S. Chairman: E. R. Callender, M.R.C.V.S. Recording Secretary: A. C. Shuttleworth, M.V.SC., F.R.C.V.S.

10 a.m. Annual General Meeting, Ladies' Veterinary Benevolent Guild (Town Hall).

2.30 p.m. Paper: "The Present Situation with regard to Food Inspection in England and Wales." By Horace Thornton, B.V.SC., M.R.C.V.S., D.V.H. Openers of discussion: Colonel Harold E. Egan, U.S. Army Veterinary Corps, and Dr. K. Seidel, Deputy Veterinary Chief of the Health Committee, Copenhagen. Chairman: H. T. Matthews, B.V.SC., F.R.C.V.S., D.V.H. Recording Secretary: William Tweed, F.R.C.V.S., D.V.S.M.

7.30 p.m. for 8 p.m. Annual Dinner (Prince of Wales Hotel).

Tuesday, September 14

9.30 a.m. Paper: "Avian Salmonellosis." By J. E. Wilson, B.SC., M.R.C.V.S., F.R.S.E. Opener of discussion: R. F. Gordon, D.SC., M.R.C.V.S. Chairman: G. O. Davies, D.V.SC., M.R.C.V.S., D.V.H. Recording Secretary: J. C. Baird, B.V.SC., M.R.C.V.S.

10.30 a.m. Ladies' excursion to the Wirral Peninsula; or Tennis Tournament in Southport.

XUM

11 a.m. Paper: "Observations on Canine Encephalitis." By A. B. MacIntyre, M.R.C.V.S., D. J. Trevan, B.SC., M.B., B.S., and R. F. Montgomerie, PH.D., B.SC., F.R.C.V.S. Opener of discussion: L. P. Pugh, B.SC., F.R.C.V.S. Chairman: D. D. Ogilvie, B.SC., M.R.C.V.S. Recording Secretary: Miss Mary Dalby, B.V.SC., M.R.C.V.S.

2.30 p.m. Divisional (Sectional) Meetings.

Afternoon Women's and Men's Golf Competitions.

8.30 p.m. Ladies' Veterinary Benevolent Guild Dance (Prince of Wales Hotel).

Wednesday, September 15

10 a.m. Closing Meeting.

11.30 a.m. Council Meeting.

12 noon Coaches leave for the visit to Evans Biological Institute, Runcorn.

SCIENTIFIC EXHIBITION

There will be a Scientific Exhibition in the Cambridge Hall during the period of Congress, which will include the following:—

By E. L. TAYLOR, D.V.SC., M.R.C.V.S., D.V.H. Displays on (*a*) The Snail and its Association with Liver Fluke; (*b*) The Efficacy of D.D.T. and Gammexane.

By JOHN FRANCIS, B.SC., M.R.C.V.S., and J. T. STAMP, B.SC., M.R.C.V.S. Joint exhibition on the Pathogenesis and Epidemiology of Bovine Tuberculosis.

By JOHN FRANCIS, B.SC., M.R.C.V.S. Graphs illustrating the Behaviour of the Sulphonamides in Domestic Animals and Methods of Control of *Str. agalactiæ* infection.

By A. B. MACINTYRE, M.R.C.V.S., D. J. TREVAN, B.SC., M.B., B.S., and R. F. MONTGOMERIE, PH.D., B.SC., F.R.C.V.S. Specimens relating to Canine Encephalitis.

By the VICTOR X-RAY CORPORATION. Demonstration of X-ray apparatus and technique and of interesting films loaned by G. C. Knight, M.R.C.V.S., A. C. Shuttleworth, M.V.SC., F.R.C.V.S., E. Brayley Reynolds, O.B.E., M.R.C.V.S., and others.

ARRANGEMENTS FOR SOCIAL EVENTS DURING N.V.M.A. CONGRESS, 1948

1. Lancashire Division Reception, Wednesday, September 8

Guests will be received by the President of the Lancashire Division and Mrs. Wright at 8.30 to 10 p.m., at the Prince of Wales Hotel, Lord Street. Running buffet. Morning dress.

2. Civic Reception, Thursday, September 9

Guests will be received by the Mayor and Mayoress of Southport at the Floral Hall, Promenade, at 8 p.m., followed by dancing until 12 midnight. Running buffet. Dress optional.

3. Visit to Liverpool University and Veterinary Field Station, "Leahurst," Friday, September 10

Coaches will leave from front of the Cambridge Hall, Southport, at 9.30 a.m., reaching University of Liverpool Quadrangle at 10.20 a.m. Welcome by the

Vice-Chancellor in the Arts Theatre at 10.30 a.m. 11 a.m. to 12.30 p.m.: Demonstrations in the Departments of Anatomy, Biology, Pathology, Bacteriology, Tropical Medicine and Surgery. 12.45 to 1.30 p.m.: Lunch at the Union. Leave Liverpool 1.30 p.m. for "Leahurst," where further demonstrations will be given until 4 p.m., when tea will be provided. Coaches will leave "Leahurst" at 5 p.m. and arrive at Southport at 6.30 p.m.

4. Excursions for Ladies, Friday, September 10

There will be a choice of two excursions:—

(*a*) Coaches will leave from front of the Cambridge Hall, Southport, at 9.30 a.m., and arrive at University of Liverpool Quadrangle at 10.20 a.m. After attending welcome by Vice-Chancellor at 10.30 a.m., proceed to Liverpool Cathedral, 11.15 to 11.45 a.m., then to Liverpool City Centre for personal exploration of shops until 12.30 p.m. Lunch at Bon Marché, 12.45 to 2 p.m. Trip on ferry to New Brighton, 2.30 to 3.30 p.m. Tea at Adelphi Hotel, Liverpool, 4 p.m., from where coaches leave for Southport at 5.30 p.m.

(*b*) Read as above as far as "Liverpool Cathedral, 11.15 to 11.45 a.m." Proceed *via* Mersey Tunnel to Chester. Lunch at Bolland's Restaurant, 12.45 to 2 p.m. Steamer trip to Eccleston Ferry and return. Explore Chester Cathedral, Walls, shops, etc. Tea at Quaintways at 4 p.m. Coaches leave from Square, opposite Cathedral, for return to Southport 5 p.m., and arrive Southport 6.30 p.m.

5. President's Reception, Friday, September 10

Guests will be received by the President, N.V.M.A., and Mrs. Anderson, at the Prince of Wales Hotel at 8.30 p.m., followed by dancing until 1 a.m. Running buffet. Dress optional.

6. Congress Photograph, Saturday, September 11

This will be taken at 12.15 p.m. in Gardens adjoining the Floral Hall, Promenade.

7. Excursion to Lakes, Sunday, September 12

Coaches will leave Messrs. Gore and Sons' Garage, Nevill Street, Southport (next door to the Coliseum) at 9 a.m.

8. Annual Dinner, Monday, September 13

The Annual Dinner will be held at the Prince of Wales Hotel at 7.30 for 8 p.m. Evening dress.

9. Ladies' Excursion to the Wirral Peninsula, Tuesday, September 14

Coaches will leave from front of the Cambridge Hall, Southport, at 10.30 a.m.

10. Golf Competitions, Tennis Tournament, Tuesday, September 14

The Golf Competitions will be arranged by Mr. D. E. Leask (for men) and Mrs. K. G. R. Kelly (for women), and the Tennis Tournament by Mrs. Hewetson.

11. Ladies' Guild Dance, Tuesday, September 14

This will be held at the Prince of Wales Hotel from 8.30 p.m. to 1 a.m.

12. Visit to Evans Biological Institute, Runcorn, Wednesday, September 15

12 noon: Two coaches will leave for Runcorn from front of the Cambridge Hall, Southport.

GENERAL ARTICLES

THE BIOCHEMICAL APPROACH TO ANIMAL DISEASE

The Fison Lectures, 1948, of the Animal Health Trust

By Professor L. SEEKLES, D.Sc.

Utrecht University

III—Real and Conditioned Deficiences of Minor Elements in Cattle

About the year 1930 on the north-east diluvial part of Holland, which is of Scandinavian origin, Sjollema for the first time established amongst the cattle conditions a deficiency due to a low copper content in the pastures.[1] From the southern diluvium these deficiencies were not reported. Whether they do occur is doubtful. This diluvial soil consists mainly of slate and other material originating from mountain areas alongside the river Rhine and the Ardennes, mixed with finely divided material of Scandinavian origin.

During recent years similar disorders in cattle have frequently occurred, although a real shortage of minor elements, e.g. copper, in the pasture was often absent.[2] This might be termed a "conditioned deficiency" and it suggests a mutual influence of the absorption and the metabolism of one or more minor elements. An interaction of minor elements with various chemical substances of the food or in the animal tissues may play a part here. The disorders are found both on the north-east diluvium and on the alluvial parts, especially on the peat bog soils of Holland. Presumably they likewise occur on the southern diluvium but definite data are not yet available. Recently the occurrence of a similar kind of hypocupræmia in cattle was reported in Great Britain by Allcroft.[3]

(1) Variety of Clinical Features: Difficulties Concerning Diagnosis

In order to establish the nature of these "conditioned deficiencies" it is necessary to inquire into the basic importance of the minor elements and their functions in the metabolic processes of the animal organism. This is primarily a scientific inquiry, as its results have deepened our knowledge of the nature of the above-mentioned disorders. On the other hand, the inquiry has its practical side, as may be illustrated by the following example.

Sometimes in a cow a conditioned deficiency of copper is accompanied by the well-known symptoms of "pica," which may be collectively called "licking sickness," like those found in a case of a real deficiency of copper. At other times the "pica" symptom is only manifested by an irregular grazing of strip

by strip of the pasture down to the roots. It seems as if the animals are on the search for something in the very young blades of grass which they apparently miss in the older blades. With horses in normal condition, that is to say not suffering from any deficiency, grazing of strips of the pasture may frequently be observed. Real and conditioned deficiency of minor elements have so far not been found with horses in Holland.

A conditioned deficiency in cows sometimes occurs which is not accompanied by " pica " symptoms. Delayed growth of young animals occurs frequently, yet anæmia, which in this connection is regarded by some as an important symptom, is far from prevalent. In the case of hæmoglobinæmia it is very questionable whether it is connected with a relative or absolute deficiency of copper. Exhaustion, scouring decreased output of milk, depressed appetite, white or greyish discoloration of the coat, shedding of the hair and a rough coat may in some years be observed in various parts of the country. It should be pointed out here that in the examination of the coat sometimes grave errors are made. Frequently symptoms of the coat which are due to a deficiency in copper are attributed to a delayed coming off of the winter coat! Sometimes a conditioned deficiency in copper is only shown by a delay in the growth and the absence of heat in the young animals, whereas the condition of the older animals is on the whole normally healthy. It may even be observed that hypocrupræmia, which in a given year is accompanied by a number of the above-mentioned clinical symptoms, in another year, though existent, causes no obvious symptoms, the animals to all appearances being in good health. This fact should make us cautious in interpreting the results of the chemical analysis of blood.

The great variation observed in Holland of the clinical features of a conditioned deficiency in copper—presumably also in other minor elements—renders it necessary to inquire into the causes of this variation. Are they to be found, first of all, or only, in the quantitative differences of the amounts of copper and other trace elements? The chemical analysis of the food, the blood and the liver of the patients does not point in this direction. Or does the individual sensitiveness of the animal play a part? Although this should not be excluded, the regional occurrence of the disorders in certain years does not support this supposition. The possible influence of the weather on the chemical composition of the grass, e.g. the amount of precipitation and the temperature, should, of course, be taken into consideration. Furthermore, farming methods, e.g. manuring, should be counted as principal factors in the differences in the clinical aspect of the symptoms. Especially during the war years the manuring of the pastures and also feeding left much to be desired. This favours the assumption that the metabolism of copper and other minor elements in plants and animals is influenced by the metabolic processes of other chemical components.

The veterinary practitioner meets diseases which are to be considered as a reflection of a number of partial effects caused by a (conditioned) deficiency of minor elements in the normal course of biochemical metabolism. It is easily understood that as a result of these effects some general symptoms, like delayed growth

and interference with other vital functions, will be observed. But it goes without saying that this effect may not be regarded as inherent to a special deficiency, e.g. of copper. It should be borne in mind that disorders of quite a different nature, like gastro-intestinal strongylosis and Johne's disease (paratuberculosis), may cause similar symptoms. A thorough clinical, parasitological and bacteriological examination should therefore never be omitted.

It should be emphasised that as a rule scouring is a prevalent clinical feature of a "conditioned deficiency" of minor elements in cattle. Evidence has been obtained that in many cases an increased movement of the intestine may give rise to a decreased absorption of minor elements and in this way may be essential to the origin of the chemical feature of low values of minor elements in the blood and tissues.

An increased intestinal movement may originate from different factors affecting the intestine, e.g. the intake of young, rank pasture grass in spring, or in the aftermath, worm infestation (gastro-intestinal strongylosis), Johne's disease and other kinds of enteritis. Over and above that, a special influence on different organs of endogenous toxins originating from gastro-intestinal autointoxication and giving rise to a mobilisation of minor elements stored in the tissues may not be excluded.

Anyhow, in a comparison of 55 normal cattle livers, we observed a significant difference between the normal content of copper of the liver and that in ten typical cases of gastro-intestinal disorders of different origin in cattle, the average copper content being 11.1 mg. and 27.1 mg. dry matter basis, respectively. In ten cases originating from a typical (peat bog) scouring area, an average value of 16.6 mg. of copper was observed, probably pointing to a decrease of copper content. (Five of those cases were calf livers, which, as a rule, show a higher copper content than those of adult animals.)

By contrast, in 189 other cases of diseases in cattle classified in three groups in which intestinal disorders were absent no significant differences concerning the copper content of the liver as compared with normal animals were observed.

The veterinary practitioner, as a rule, will not be able fully to differentiate the above-mentioned partial effects. The inevitable consequence is a certain vagueness in making a diagnosis and uncertainty in therapeutic planning.

The experience of recent years has shown that, as a rule, the veterinary practitioner—and less so, naturally, the breeding or agricultural expert—is not equal to arriving, as a result of his observations, at a sure diagnosis of a (conditioned) deficiency in minor elements. To do this with a reasonable chance of success, he needs the assistance of a chemist who can help him with the analysis of the food, the blood and the tissues of the animals.

So far, mainly the composition of pastures and the symptoms of disease in plants and in herbivorous animals have received attention in the study of the functions of minor elements. This research is of the utmost value, but for a knowledge of the origin of the disorders in plants and in animals resulting

from a relative deficiency or excess of minor elements, further research is required into what happens between the absorption of the food and the moment in which pathological disorders in the plant or animal become manifest. The study of the real basis of these disorders is the work of the biochemist, as the processes of intermediate metabolism are of a biochemical nature. Only a thorough biochemical examination of the reactions, progressing step by step in cells, tissues and organs, can lead to rational diagnosis and an effective fight against disease.

For a number of years, in the Laboratory for Veterinary Biochemistry of the University of Utrecht, researches have been carried out on the occurrence of minor elements—especially copper, cobalt, manganese and, incidentally, molybdenum and selenium—in pasture grass and in the blood and liver of cattle and horses. Our results are based on the clinical and chemical examination of many hundreds of patients. It was found necessary to extend our research from minor elements to other chemical components so as to acquire an insight into the behaviour of minor elements in the processes of metabolism. The results obviously do not yet present a complete picture, but some of the conclusions may be submitted here.

(2) Mode of Action of Minor Elements in Intermediate Metabolism

Recently, we showed that the ions of minor elements may act on metabolic processes in the animal body in two different ways.[2] Firstly, they may be taken up in the molecules of enzymes, vitamins and hormones, these complexes acting as bio-catalyzers in the processes of intermediate metabolism. Secondly, they may act as activators of enzymes and in this way exert an influence on metabolic processes.

A general survey of minor elements acting on enzymes in those two different ways is given in Table I.

TABLE I

ENZYME SYSTEMS INFLUENCED BY MINOR ELEMENTS

Enzyme	Where Active	Action	Minor Element
A. *Synthetic or breakdown reactions*			
1. d-Peptidase	Animal organs, blood serum, plants, bacteria	Peptides → amino acids	Mn, Co, Fe, Zn, Mg.
2. Arginase	Muscle, liver, yeast	Arginine → ornithine + urea	*Mn*, Co, Ni, Ca, V.
3. Lipase	Pancreas, stomach, intestine, bacteria, plant seeds	Fats → fatty acids	Ca, Mn.
4. Zymase	Yeast	Glucose → Alcohol + CO_2	Mn.
5. Carboxylase	Yeast	Pyruvic acid → acetaldehyde + CO_2	Mn, *Mg*, Zn.
6. Carbonic anhydrase	Blood	H_2CO_3 → CO_2 + H_2O	***Zn***.

ENZYME SYSTEMS INFLUENCED BY MINOR ELEMENTS

TABLE I (continued)

Enzyme	Where Active	Action	Minor Element
A. *Synthetic or breakdown reactions*			
7. Phosphatase	Various organs	Decomposes organic phosphates	Mg.
8. Phosphate transportase	Yeast	Transporting phosphate between diphosphoglyceric acid and adenosine diphosphate	Mg, Mn.
B. *Energy processes (oxido-reduction reactions)*			
1. Respiratory enzyme	In living cells using oxygen	Transference of oxygen in tissues	*Fe.*
2. Cytochromes	In cells of micro-organisms, higher plants and animals	Ditto	*Fe,* Mn.
3. Hæmoglobin	Blood (r.b.c.), muscle pigment	Ditto	*Fe.*
4. Hæmocyanin	Certain crustacea	Ditto	*Cu.*
5. Peroxidase	Many plant and animal tissues	Liberates O_2 from peroxides	*Fe,* Mn.
6. Catalase	Almost all tissues	$H_2O_2 \rightarrow H_2O + O$	*Fe.*
7. Pyruvic acid dehydrogenase	Brain	Oxidises pyruvic acid	Mg, Mn.
8. Iso-citric acid dehydrogenase	Heart muscle, liver, kidney, adrenals	Iso-citric acid → α keto-β carboxy glutaric acid	Mn, Mg.
9. Phospho-lipoid oxidising enzyme	Kidney, brain	Oxidises phospholipoids	V.
10. Enolase	Yeast	Oxidises phosphoglyceric acid to phospho-pyruvic acid	*Mg,* Mn, Zn.
C. *Detoxication reactions*			
1. Diamino oxidase (Histaminase)	Mucous membrane of intestinal tract, etc.	Oxidation of diamines, histamine	Co.
2. Tyrosinase	Mushrooms	Oxidation of tyrosine, p-cresol, pyro-catechol	*Cu.*
3. Poly-phenol-oxidases	Mushrooms, potatoes, milky juice of plants	Oxides p-cresol, pyrocatechol, tyrosine, pyrogallol, hydroquinone, p-phenylamine diamine	*Cu.*
D. *Transference of nervous stimuli*			
Cholinesterase	Blood serum, blood cells, torpedo	Acetylcholine → choline + acetic acid	Mn, Mg, Ca, Ba.

Elements in italics are bound to enzyme complexes.

Two facts should be noticed. In many cases an optimal concentration of the minor elements is shown to exist for the activation of enzyme action. Evidence has been obtained that both less and more of these elements lowers the velocity of enzyme action. The latter conditions may be compared with the conditions of deficiency and intoxication respectively of minor elements in the animal body. Furthermore, the indication that minor elements act through their implication in enzymic activity indicates that the pH of the body tissues will have some influence on the action of minor elements in the animal organism.[4]

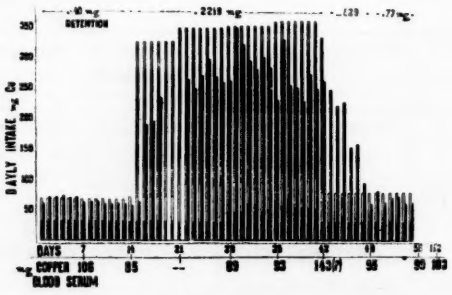
Table I shows that four kinds of processes of fundamental importance for normal life are subject to the action of trace elements, viz.: (1) the synthesis and destruction of tissue elements (mainly protein); (2) energy processes (oxido-reductions); (3) detoxication of poisons of endogenous origin; (4) the regulation of nervous stimuli.[2]

This opens a new outlook on the genesis and therapy of several diseases, e.g. "licking sickness" (pica) in animals, ketonæmia in cattle, avitaminosis and hypovitaminosis B_1 and C, autointoxication in men and animals, and perhaps even on the problem of carcinoma.

(3) State and Distribution of Copper in the Animal Body

(a) *Experiments with Cattle*:

The level of copper (and other elements) in the blood is often brought into relation with "deficiency," but, when doing so, many difficulties arise. This led us to carry out balance trials in which copper sulphate (and salts of other trace



GRAPH 1. BALANCE TRIAL USING COPPER SULFATE ADDED TO THE FOOD.
(EXPERIMENT WITH A NORMAL HEIFER)

elements) was administered by mouth and to experiments in which copper, bound chemically in different ways, was injected intravenously. Attempts have been made to obtain information about the extent of saturation of body tissues before and after treatment. Graph 1 shows the results of a *balance trial* in which copper sulphate was added to normal food (hay and concentrates). The experiment was carried out with a normal heifer.

In the period previous to the administration of copper sulphate, the intake of copper was shown to be almost in balance with the excretion of copper in the fæces. Only traces of copper left the body by way of the kidneys.

As might be expected, the excretion of copper in the fæces increased during the period of the addition of copper, the absorption of added copper not being complete. The (alkaline) urine was shown to contain only traces of copper. No rise in the copper level of the blood occurred. This points to a condition of non-saturation of the body tissues.

During a fortnight prior to the administration of copper sulphate, a retention of 40 mg. of copper was calculated. However, it seems to be doubtful whether this was a real retention. The traces of copper leaving the body by way of the kidneys were not taken into account. In the three weeks' period during which copper sulphate was given together with the following six days, a retention of 1,590 mg. of copper could be established, being about 30 per cent. of the quantity of copper added. (Three days had to be excluded from calculation.) In the next seven-day period, retention of 77 mg. of copper was recorded. Apparently about 50 mg. may be considered to be a real retention in this period.

To secure information on *the concentration* of copper in the animal body, determinations have been carried out on 55 samples of liver from normal cattle aged 1½ years and older. The samples were collected in war-time. The content of copper was shown to range from 5 to 194 mg. per kg. dry matter basis. A classification of the results is given in Table II.

TABLE II.

COPPER CONTENT (MG. PER KG. DRY MATTER BASIS) OF 55 SAMPLES OF LIVER FROM NORMAL FEMALE CATTLE.

(*In collaboration with R. Bijkerk*)

Mg. Cu	5-15	16-25	26-35	36-45	46-55	56-65	66-75	76-85	86-95	—	186-195
No. of cases	34	4	3	3	1	2*	3	2	2		1†
Percentage	62	7	5	5	2	4	5	4	4		2

* One sample came from a calf (½ year of age).
† The sample came from a bull.

Table II shows that about 60 per cent. of the livers examined had a copper content ranging from 5 to 15 mg. per kg. of dry matter and about 40 per cent. contained more copper values ranging up to 91 mg. for a cow and 194 mg. per kg. for a bull (one case only).

Thus, in 60 per cent. of the cases examined the extent of saturation of the liver seems rather low. This may account for the relative stability of the copper content of the blood following an oral administration of copper sulphate.

A new series of experiments furnishes further information on the absorption of copper by the liver following intravenous administration. The experiments were carried out using *radioactive copper salts*.[5] Three copper salts—copper acetate, copper glycinate and the sodium salt of copper allylthioureabenzoic acid ("Ebesal")—were injected, using calves with a body weight of about 30 kg. The copper salts had been irradiated by a neutron generator (Ra-Be preparation) during 24 hours or longer. The acquired radioactivity of the copper—measured

by means of a sensitive Geiger-Müller counter—enabled us to measure the rate of absorption of the various copper salts by the liver.

The relatively slight radiation of the copper salts and the rather short half-value time (13h) of the radioactive copper isotope forced us to inject as large quantities as could be tolerated by the animals. Table III shows the results.

TABLE III.

ABSORPTION OF RADIOACTIVE COPPER BY THE LIVER FOLLOWING INTRAVENOUS INJECTION IN CALVES.

(In collaboration with E. Havinga and R. Bijkerk)

	Cu-acetate	Cu-glycinate	Cu-Na-allylthiourea-benzoate ("Ebesal")
Amount of Cu in the solution injected	0.73 g.	0.56 g.	0.78 g.
Time from first injection till slaughter	3½ h.	3¾ h.	3¾ h.
Amount of injected Cu recovered from the livers ...	13.7 ± 1.4% (0.10 g.)	16.1 ± 1.1% (0.09 g.)	24.5 ± 1.1% (0.19 g.)

Evidently there is a correlation between the "solubility" of the copper compound used for injection and the rate of its absorption by the liver.

The difference between Cu-acetate and Cu-glycinate is only slight; probably in both cases copper is almost equally adsorbed by compounds of the blood and transported to the tissues, including the liver. This is corroborated by our finding that the lethal dose of each of these two salts when injected intravenously contains the same amount of copper.

For "Ebesal," the lethal dose proved to be higher and the amount of Cu taken up by the liver was greater.

These experiments may explain the results mentioned in Graph 1 and, consequently, point to a condition of non-saturation of the liver.

As a conclusion, we might give it as our opinion that injection of ionogenic compounds (Cu-acetate) or not too firmly bound complex salts (Cu-glycinate) will not show much difference in pharmacological and therapeutical effects. Compounds which give (practically) no Cu-ions, like "Ebesal," will behave in a different way, viz., being less toxic and resulting in a higher Cu-content of the liver (and probably a lower one in other organs) after injection.

(b) *Experiments with Horses*:

In normal horses the copper content of blood serum is somewhat higher than in cattle. Observations made in this laboratory give round values of 100-130 mg. per cent. Mares in foal may show much higher copper values.

Copper glycinate repeatedly administered by intravenous injection in quantities of 30 mg. of copper did not give rise to any regular increase of the copper content of the blood serum. The copper level of the blood serum seems to vary somewhat irregularly. Apparently the excess of copper injected is rapidly transferred to the tissues (Table IV).

TABLE IV.

INTRAVENOUS INJECTION OF COPPER GLYCINATE (30 mg. OF Cu PER INJECTION) IN HORSES.

(*In collaboration with E. Havinga*)

		Days										Weeks							
		0	1	2	4	6	7	12	13	16	19	1	2	3	4	5	6	7	8
Gelding No. 1	Injection														↓		↓	↓	
	per cent. of Cu in blood serum	144	87	91	—	135	—	88	96	160	131	—	—	—	100	127	139	112	120
Gelding No. 2	Injection	↓											↓	↓	↓	↓	↓	↓	
	per cent. of Cu in blood serum	82	123	—	159	—	153	—	—	—	—	153	178	97	124	110	117	181	122
Mare	Injection	↓											↓	↓	↓	↓	↓	↓	
	per cent. of Cu in blood serum	121	103	—	194	—	169	—	—	—	—	169	184	122	135	111	141	155	130

To obtain information on the *extent of saturation of the liver,* determinations of copper were carried out in samples of liver from normal and nearly normal horses (Table V).

TABLE V.

COPPER CONTENT (mg. PER kg. OF DRY MATTER) OF SAMPLES OF LIVER FROM HORSES.

(*In collaboration with R. Bijkerk*)

Cu in mg.		11-15	16-20	21-35	26-30	31-35	Extreme values	Average value	Total No. of cases
Normal (c.q. killed by accident)	No.	13	16	5	2	1	13-32	18.5	37
	%	35	43	13	6	3			
Probably normal (c.q. light disorders)	No.	13	15	8	1	0	12-28	17.6	37
	%	35	41	21	3	—			
Normal, in foal or shortly after deliverance	No.	6	3	1	0	0	14-21	16.1	10
	%	60	30	10	—	—			
Surgical patients (lameness, etc.)	No.	3	25	4	1	0	14-29	18.8	33
	%	9	76	12	3	—			
New-born foal	—	—	—	—	—	—	223	—	1

The copper content of the liver of normal adult horses shows considerably less variation than the copper values of cattle liver. The maximum of the cases examined is shifted to somewhat higher values in comparison with cattle. Values up to 10 mg. of copper per kg. of liver (dry matter basis) which were observed in cattle in 51 per cent. of the cases examined did not occur in horses.

Evidence has been obtained from the examination of a small number of livers, viz., ten samples from mares in foal or shortly after foaling, that there is a shift towards lower copper values in pregnant mares. In surgical patients there seems to be a tendency of a shift in the opposite direction.

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In a new-born foal the copper content of the liver was shown to be 10-15 times higher than in adult horses.

(4) Manganese in Relation to Grass Tetany and Sterility in Cattle: The Manganese Content of Pasture Grass and Hay in the Netherlands

a. *Manganese in relation to grass tetany*

The observations of Blakemore, Nicholson and Stewart suggesting that in Lincolnshire "lactation tetany" in cattle occurs under conditions in which the manganese-intake is likely to be at its maximum,[6] forced us to make an investigation on the possibility of a correlation between the occurrence of grass tetany and a high manganese content of pasture in the Netherlands. If a correlation exists it would point to a third example of a disease among animals due to excess of minor elements: the first two examples being alkali disease (Se) and the scouring of cattle on the "teart lands" of Somerset (Mo).

Just as in England, in the Netherlands the majority of cases of grass tetany occur when cattle are first turned out to pasture in the spring. A second though less severe outbreak is seen in August and September on the aftermath. The latter kind of grass tetany has been always known in Holland. It was found to occur especially in an area alongside the river *Ouden Rijn*. The soil in the affected area consists of river clay, at some places mixed with sand or peat bog. The results are given in Table VI.

TABLE VI.

MANGANESE CONTENT OF PASTURE GRASS IN RELATION TO THE OCCURRENCE OF GRASS TETANY.

	Manganese content of pasture grass mg. p. 100 g., dry matter basis
Grass tetany in spring: 40 affected farms	4.1— 19.5
Grass tetany in autumn: 9 affected farms	3.3— 27.6
No grass tetany: 15 farms	5.5— 17.6
"Lactation tetany" in Lincolnshire (Blakemore & coll.):	
11 affected farms	54.0—132.0
No grass tetany (Lincolnshire): 12 farms (24 samples)	1.6— 9.2

A relation of the intake of manganese to the occurrence of grass tetany as suggested by Blakemore *et al.* could not be established in the Netherlands.

b. *Manganese in relation to sterility*

A deficiency of manganese is related to sterility in rodents. In young rats kept on a manganese deficient diet growth seems to be normal.[7] The females show a normal cycle and produce normal litters. However, the animals were unable to suckle the new-born young. In males kept on a manganese deficient ration a degeneration of the testes was observed within 100 days, causing complete sterility. In recent observations rats showed the development of features of manganese deficiency following the feeding of a ration rich in vitamin B_1.[8] The F_1 generation showed disturbances of fertility and lactation and, moreover, cannibalism. In young mice kept on a manganese-deficient food growth was shown to be stunted, ovulation stopped and heat seldom occurred.[9]

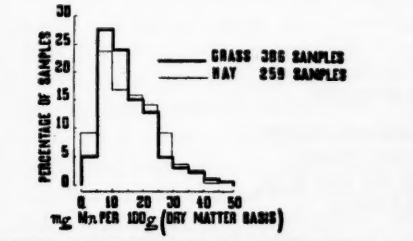
It remains to be seen whether the frequent occurrence of sterility in male and

female cattle, not due to anatomical and pathological disorders, might be connected with a (relatively) manganese deficiency.[2 10] It has already been shown that the manganese content of pasture grass and hay in the Netherlands may vary considerably, depending on the condition of the soil.[2] *Sometimes the manganese values are as low as* 2 *mg. per* 100 *g.*, dry matter. These low values are observed on soils consisting of heavy clay showing high pH values. The intake of manganese is very low in these cases. Moreover it seems to be doubtful whether the absorption of manganese in the gastro-intestinal tract is a complete one.

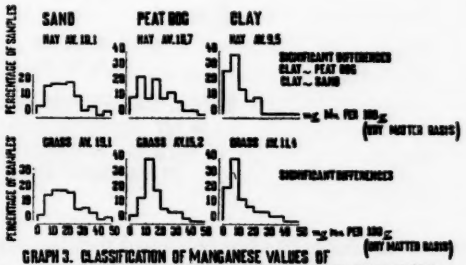
Thus if sterility due to absolute or relative manganese deficiency does occur in the Netherlands this disorder may be expected to occur in areas of clayish soils of alkaline reaction. Further research is progressing.

c. *The occurrence of manganese in pasture grass and hay in the Netherlands*

A general survey of the occurrence of manganese in 386 samples of young pasture grass and 259 samples of hay from different parts of the country and in 60 samples of hay from the province of Friesland is given in Graphs 2, 3 and 4.



GRAPH 2. MANGANESE CONTENT OF PASTURE GRASS AND HAY WITHOUT MENTIONING THE KIND OF SOIL, MANURING AND THE CONDITION OF THE PASTURE (IN COLLABORATION WITH J.T.L.B.RAMEAU)



GRAPH 3. CLASSIFICATION OF MANGANESE VALUES OF PASTURE GRASS AND HAY DEPENDENT ON THE KIND OF SOIL (IN COLLABORATION WITH J.T.L.B.RAMEAU)

Graph 2 shows that in about 50 per cent. of the grass samples examined a manganese content of between 5 and 15 mg. per cent. (dry matter basis) was observed. About 30 per cent. of the samples show values between 15 and 25 mg. Thus, altogether 80 per cent. show a manganese value up to 25 mg. The average manganese value of 386 samples of pasture grass is 15.8 mg.

The samples of hay show a similar distribution of manganese values, 70 per cent. with a variation between 5 and 25 per cent., the average manganese value of 259 samples of hay being 16.1 mg.

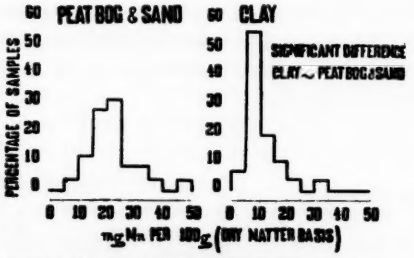
A classification of manganese values of the same samples of pasture grass and hay dependent upon the kind of soil is given in Graph 3.

No significant difference was shown to exist between the manganese contents of the pasture grass and the hay from any of the three (sandy, clayish or peat bog) soils.

Grass from sandy soils, however, shows a higher manganese value than grass from peat bog soils. Grass from clayish soils has the lowest manganese content. The average values are 19.1, 15.2 and 11.4 mg., respectively. *The differences were shown to be significant.*

So far as hay is concerned, a higher manganese content was observed in hay from sandy and peat bog soils in comparison with clayish soils. The average values are 18.1, 16.7 and 9.5 mg., respectively. The difference between the first two values is not significant, but the difference between those values and the third is significant.

A similar result was observed with 60 samples of hay from the province of Friesland (Graph 4).



GRAPH 4. CLASSIFICATION OF MANGANESE VALUES IN HAY ORIGINATING FROM THE PROVINCE OF FRIESLAND DEPENDENT ON THE KIND OF SOIL (IN COLLABORATION WITH J.T.L.B. RAMEAU)

Evidence has been obtained that the pH value of soils plays an important part in the absorption of manganese by pasture grass. The lower the pH value of the soil the more manganese is absorbed by the grass. This may explain the differences observed between the manganese values of pasture grass (and of hay) from different soils.

Dr. Rameau, by performing a critical survey of the results obtained in the Utrecht Laboratory concerning chemical composition, kind of soil, manuring and condition of the pastures, made a series of supplementary observations.

The influence of the pH value of the soil is indicated by the significant difference which was established between the manganese content of the pasture grass from sandy areas when it was or was not manured with chalk. The addition of chalk lowered the manganese content of the pasture grass. In hay the differences were shown to be not significant in this respect.

Manganese values of pasture grass from sandy soils not treated with chalk showed a gradual decrease when passing from the northern to the southern provinces of the Netherlands. It is a well-known fact that in plough land of sandy areas the pH value increases in the same direction. In hay those differences are hardly significant.

On clayish soil a considerable increase of the manganese content of young pasture grass was observed following manuring with thomas phosphate containing 6 per cent. manganese, the average values being 8.6 and 19.0 mg., respectively. In hay the difference is not significant. On sandy and peat bog soils the differences of manganese values of young pasture grass and hay following manuring with thomas phosphate were not significant.

Evidence has further been obtained that rank young pasture grass from sandy soils has a lower manganese value than ordinary young grass.

(5) Supplementary Research on Copper, Cobalt, Molybdenum and Selenium
(*In collaboration with F. W. J. van Haaren and J. de Wael*)

Low values of *copper* in pasture grass and hay formerly reported from the north-eastern diluvial part of the Netherlands were observed in alluvial parts in recent years. Values as low as 3-5 mg. of Cu per kilogram (dry matter basis) are not exceptional. However, in the majority of the cases examined normal values of 10-25 mg. were shown to occur. As was already pointed out, low values of copper in the blood serum of cattle grazing on these pastures pointing to a condition of " conditioned deficiency " were repeatedly recorded.

Cobalt was determined in pasture grass, hay and livers of cattle in numerous cases. In pasture grass and hay originating from alluvial soils values of about 300-350γ per kg. (dry matter basis) were found to be quite normal. Cobalt values of grass and hay from diluvial soils may be considerably lower, e.g., half of the amount mentioned and even lower. Features of cobalt deficiency are repeatedly reported in these areas. Evidence has been obtained that the affected animals—cattle and goats—may show a favourable response to the oral administration of cobalt or to the addition of a mixture of cobalt and copper. The liver of normal cattle was shown to contain about 300γ-400γ of cobalt per kg. dry matter basis. The liver of cattle from affected areas may show lower values, e.g., 120γ.

Molybdenum and selenium were occasionally determined in pasture grass, hay, drinking water and soil from farms on which cattle showed severe symptoms of shedding of hair and acetonemia. In all cases examined no excess of molybdenum or selenium was found.

Discussion

The great variety of clinical features observed in cases of a real and conditioned deficiency or in cases of an excess of minor elements may easily be understood, assuming a variety of action on different enzyme systems acting in the majority of the processes of intermediate metabolism. The veterinary practitioners and breeders meet diseases which are to be considered as a reflection of a number of partial defects in the normal course of biochemical processes in the animal body. As a result of these defects some general symptoms like stunted growth and interference with other vital functions will be observed. However, as a rule these defects may not be regarded as inherent to a special deficiency or excess of a special minor element.

In many cases of " conditioned deficiency " of minor elements scouring is a prevalent clinical feature. Evidence has been obtained that in many cases increased

movement of the intestine may give rise to a decreased absorption of minor elements and in this way may be essentially concerned with the origin of the chemical features of low values of minor elements, e.g., copper in the blood and in the tissues (liver).

Increased intestinal movement may arise from different factors affecting the intestine, independent of the content of minor elements in the food, e.g., the intake of proteins and other components of young rank pasture grass in spring and on aftermath, worm infestation (gastro-intestinal strongylosis), Johne's disease (paratuberculosis) and other types of "enteritis."

In cattle the copper content of the liver was shown to vary between 5 and 194 mg. per kilogram dry matter basis. In 62 per cent. of the cases observed the copper content did not exceed 5-15 mg., showing a very low extent of saturation of the liver. This may account for the relative stability of the copper content of the blood following a daily administration of copper sulphate by mouth to a heifer for a period of three weeks. In this balance trial about 1.6 g. of copper, that is to say about 30 per cent. of the copper added to the food, was retained in the tissues. That the liver is of much importance in the accumulation of copper is shown by experiments in which radioactive copper salts were administered to calves by the intravenous route.

In horses the copper content of the blood serum is somewhat higher than in cattle. Intravenous injections of copper glycinate were shown to give rise to a rather irregular variation of the copper content of the blood serum. The copper content of the liver of normal adult horses shows considerably less variation than the copper values of cattle liver, the limits observed being 13 and 32 mg. per kilogram dry matter basis. The maximum of the cases examined is shifted to higher copper values compared with those of cattle. Apparently in pregnancy there is a shift of the copper content of the liver to lower values.

A relation between the intake of manganese and the occurrence of grass tetany in cattle in the Netherlands could not be established.

A general survey of the manganese content of 645 samples of pasture grass and hay from different soils gives rise to several conclusions. Significant differences were shown to exist between the manganese values of pasture grass from sandy, peat bog and clayish soils, the average values being 19.1, 15.2 and 11.4 mg. per 100 g., respectively, dry matter basis. Presumably the pH value of the soil is of considerable importance here, more alkaline soils showing the lowest manganese values of the pasture. No significant difference was observed between the manganese values in hay from sandy and peat bog soil, respectively, but a significant difference was shown to exist between these two kinds of hay and the hay from clayish soils.

The occurrence of sterility in cattle originating from manganese deficiency may not be excluded. This kind of sterility may be expected to occur in areas of clayish soils of alkaline reaction. No correlation between the occurrence of grass tetany and manganese values of pasture grass was found.

XUM

Summary

1. Real and conditioned deficiency of copper in cattle was observed in the diluvial and alluvial parts of the Netherlands. So far a shortage of cobalt in cattle was mainly observed in diluvial areas.
2. Pasture grass from sandy soils shows a higher manganese value than that from peat bog soils. Pasture grass from clayish soils shows the lowest manganese content. The manganese content of hay from clayish soil is significantly lower than in hay from sandy and peat bog soils. The pH value of soils plays an important part in the absorption of manganese by plants, the lower the pH the more manganese being absorbed. Manuring of clayish pastures with thomas phosphate containing much manganese causes a considerable rise of the manganese content of the pasture grass. Sterility due to manganese deficiency in cattle may not be excluded in clayish areas showing a high pH value of the soil. No relation could be established between the occurrence of grass tetany in cattle and the manganese content of the pasture.
3. Minor elements act as bio-catalysers of enzyme action in fundamental processes of intermediate metabolism. This accounts for the great variety of clinical features in diseases due to a deficiency of minor elements.
4. In many cases scouring is a prevalent clinical feature. Increased movement of the intestine gives rise to a decreased absorption of minor elements and in this way may be essential to the origin of low values of minor elements in the blood and the tissues (liver). Increased intestinal movement may originate from different chemical and microbiological factors affecting the intestine.
5. As a rule cattle liver shows a low extent of saturation with regard to copper and a wide variation in copper content. In horse livers there is less variation of copper content and a higher average value of copper.
6. In a three weeks' balance trial with a heifer about 30 per cent. of the copper sulphate added to the food was retained in the body. Radioactive copper injected intravenously into calves was stored in the liver to a considerable extent, e.g., *in maximo* 24.5 per cent. of the quantity injected in a period of 3¾ h. Evidence has been obtained that copper injected intravenously into horses is rapidly transferred to the tissues in the same way.
7. So far no evidence has been obtained that molybdenum or selenium plays a part in the setting up of diseases in farm animals in the Netherlands.

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A SURVEY OF BOVINE SALMONELLOSIS IN MID AND WEST WALES

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Case No. 4.—The description of the following outbreak is included to illustrate the finding that when a clinical case occurs in a herd several animals may, in fact, be suffering from transient infections which do not develop into clinical cases showing the recognised symptoms. Another feature illustrated by this outbreak is the considerable "lag" phase which may be found between the primary infection and the development of gastro-intestinal symptoms. Finally, a common source of infection is indicated.

Result of periodic tests of animals pos

No. of animal	9-1-47 "H"	9-1-47 "O"	9-1-47 Fæces	21-3-47 "H"	21-3-47 "O"	21-3-47 Fæces	5-5-47 "H"	5-5-47 "O"	5-5-47 Fæ
Group 1 Adults									
21	1/1280	1/320	*S.d.*	1/1280	1/320	*S.d.*		Sold	
22	1/1280	1/640	*S.d.*		Sold				
23	1/1280	1/320	*S.d.*	1/1280	1/320	*S.d.*	1/640	1/160	*S.*
26	1/1280	1/1280	*S.d.*	1/1280	1/640	*S.d.*		Sold	
44	NS	NS	NS	1/640	1/640	*S.d.*	1/1280	1/320	*S.*
49	NS	NS	NS	1/640	1/320	*S.d.*	NS	NS	N
52	NS	NS	NS	1/1280	1/640	*S.d.*	NS	NS	N
Group 2 Adults									
28	1/160	1/40	*S.d.*	NS	NS	NS	1/160	1/20	Ne
37	NS	NS	NS	Neg.	1/10	*S.d.*	NS	NS	N
38	NS	NS	NS	1/10	1/20	*S.d.*	1/20	1/10	Ne
50	NS	NS	NS	Neg.	1/20	*S.d.*	Neg.	1/10	Ne
Group 3 Calves									
2	1/1280	1/640	*S.d.*	1/320	1/80	Neg.	1/160	1/20	Ne
17	1/1280	1/80	*S.d.*	1/20	1/10	Neg.	NS	NS	N
3	1/1280	Neg.	*S.d.*	1/1280	Neg.	*S.d.*	1/640	Neg.	Ne
4	1/320	Neg.	*S.d.*	Neg.	1/20	*S.d.*	Neg.	Neg.	Ne
5	Neg.	Neg.	*S.d.*	Neg.	1/20	Neg.	NS	NS	N
7	1/10	Neg.	*S.d.*	Neg.	1/20	Neg.	NS	NS	N

NS = Not sampled. Neg. = Negative.

History.—On May 10, 1946, four Shorthorn cows suddenly developed febrile symptoms with temperatures varying between 106-107 deg. F., cessation of milk yield and inappetance. After treatment with sulphonamides and febrifuges, the temperatures dropped to normal within 48 hours and the milk yield returned. On May 31, three weeks later, one of these animals again developed a febrile reaction

Continued from page 266, August, 1948

and the following day the acute dysentery and depression associated with *S. dublin* infection appeared. By June 3, dehydration and wasting were pronounced and milk yield had completely ceased. Abortion of a five-months calf occurred on June 5, and an *anærogenic* strain of *S. dublin* was recovered from the heart blood, lung, spleen and fourth stomach of the fœtus. On June 3, the fourth day of clinical illness, an examination was made of the blood serum of all the animals for agglutinins to *S. dublin* and of fæces and milk for the specific organism. The results obtained are shown in detail in Table 3. An *anærogenic strain* of *S. dublin* was isolated from the fæces and/or milk of seven of the 13 cows in the milking herd. At a subsequent herd test on July 22, however, *S. dublin* was recovered only from the fæces and milk of the animal clinically affected in June (Animal No. 3, Table 3).

Cow No. 3 has subsequently been sampled on six occasions, the last being on

II
No. 3
at the primary examination of the herd

2-6-47			7-7-47			1-10-47		
"H"	"O"	Fæces	"H"	"O"	Fæces	"H"	"O"	Fæces
1/640	1/160	*S.d.*	1/640	1/160	*S.d.*	1/640	1/80	*S.d.*
1/1280	1/160	*S.d.*	1/1280	1/160	*S.d.*	1/1280	1/160	*S.d.*
1/1280	1/320	*S.d.*	1/1280	1/320	*S.d.*		Sold	
NS	NS	NS	1/1280	1/160	*S.d.*		Sold	
1/160	1/20	Neg.	1/80	1/10	Neg.	1/80	1/10	Neg.
NS	NS	NS	Neg.	Neg.	Neg.	NS	NS	NS
NS	NS	NS	1/20	Neg.	Neg.	NS	NS	NS
NS	NS	NS	1/10	1/10	Neg.	1/10	Neg.	Neg.
1/160	1/10	Neg.	1/160	1/10	Neg.	1/40	1/10	Neg.
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	1/20	Neg.
1/1280	Neg.	Neg.	1/1280	1/10	Neg.	1/640	1/20	Neg.
NS	NS	NS	Neg.	Neg.	Neg.	Neg.	1/10	Neg.
NS	NS	NS	NS	NS	NS	NS	NS	NS
NS	NS	NS	NS	NS	NS	NS	NS	NS

S.d. = *S. dublin.*

March 2, 1948. At all tests an *anærogenic* strain of *S. dublin* has been isolated from the fæces.

In view of the fact that *S. dublin* was recovered from the milk of this animal on July 22, i.e., after apparent clinical recovery, and in view of the important public health implication of this finding, further milk samples were taken from this animal during one week in October, 1946. Quarter samples of milk were

taken on three occasions before the afternoon milking. The udder was washed and dried and the teats wiped in cotton wool soaked in 50 per cent. alcohol. Ten c.c. of milk were drawn from each quarter into 1 oz. McCartney bottles containing 15 c.c. selenite broth. The broths were incubated at 37 deg. C. for 18 hours when a sub-culture was made on desoxycholate citrate agar. Plates were read after incubation at 37 deg. C. for 24 hours. At two out of the three tests one of the quarter samples yielded *S. dublin*. Cow No. 3 has been shown to have remained a carrier for 21 months. No evidence of transmission of infection to either adult cattle or calves has been found during the period of observation.

TABLE III

Result of examination of the whole herd on June 3, 1946

No. of Animal	Fæces	Milk	Blood titre "H"	Blood titre "O"
1	Negative	Negative	1/10	1/20
2	Negative	*S. dublin*	1/20	1/20
3	*S. dublin*	*S. dublin*	1/80	1/40
Affected				
4	*S. dublin*	*S. dublin*	1/10	1/10
5	Negative	Negative	1/10	1/20
6	*S. dublin*	*S. dublin*	1/80	1/20
7	Negative	Negative	1/20	1/10
8	*S. dublin*	Negative	1/10	1/10
9	Negative	Negative	1/10	1/20
10	Negative	Negative	1/10	1/10
11	Negative	*S. dublin*	1/20	1/20
12	Negative	*S. dublin*	Negative	1/20
13	Negative	Negative	1/20	1/40

Case No. 5.—Dry cows and a bunch of seven maiden heifers were running together during the summer of 1945 in a meadow bounded on one side by a river. In July one of the adult cows developed acute dysentery and died. No bacteriological diagnosis was made as to the cause of death. On October 13 three of the maiden heifers showed profuse dysentery and acute depression. One died within 48 hours of the onset of symptoms. *S. dublin* was isolated from the fæces.

On April 24, 1946, six months subsequent to the occurrence of the clinical cases, an examination was made of the blood serum and fæces of all members of the herd. The only animals to show a high titre to *S. dublin* were the two heifers, recovered from clinical infection. The fæces of both yielded a profuse growth of *S. dublin*.

The six heifers have subsequently been tested at intervals. The results obtained are shown in Table 4. The blood titre in the two heifers has remained at a high level and the fæces have yielded *S. dublin* at all examinations. Further reference to the table indicates that *S. dublin* was recovered from the fæces of the

four remaining heifers in the bunch at one or other of the tests. Not one of the four was positive on fæces examination on more than two occasions.

At a visit to this farm on July 15, 1946, it was found that the bunch of heifers, including the two chronic carriers, had for some 14 days been running with six dry cows and younger heifers at grass. Samples of fæces were taken from the six " in-contact " animals to determine whether any transmission of *S. dublin* had occurred. The organism was isolated from the fæces of one dry cow and one heifer, i.e., from two of the six animals.

During a further visit to the farm on April 21, 1947, it was found that one of the six heifers had recently calved. The heifer concerned, No. 74, was not one of the chronic carriers but *S. dublin* had been recovered from her fæces at a test in May, 1946. Blood and fæces samples were taken from the calf. The blood serum showed high flagellar agglutinins to *S. dublin* and the organism was recovered from the fæces. The calf showed no sign of clinical illness.

On July 26, 1947, approximately two years after the first case to occur on this farm, and 21 months following the cases in the heifers, the owner bought and brought on to the farm a first-calving Friesian heifer. On August 12, i.e., 17 days later, the animal developed typical symptoms of salmonellosis and *S. dublin* was recovered from the fæces. The animal had been allowed to graze with the remainder of the herd, including the chronic carrier animals. A number of points of interest arise out of the observations made on this herd:—

1. The animals recovered from clinical infection have remained carriers for at least two years.

2. Animals grazing with carrier animals are exposed to infection as shown by the isolation of the organism in their fæces.

3. The isolation of *S. dublin* from four heifers at one or other of the tests suggests that they represent either " contact carriers " or " latent carriers " excreting the organism intermittently.

4. The isolation of the organism from the fæces of the calf of one of the bunch of heifers, together with the demonstration of agglutinins in the serum, confirmed observations made in other herds that adult carrier animals constitute a reservoir of infection for calves.

Case No. 6.—An adult cow in this herd developed typical symptoms of salmonellosis on November 22, 1945. On December 12, she aborted, finally recovered and was eventually fattened for slaughter. At the time of illness fæces samples were taken from various members of the herd and one apparently normal animal was found to be excreting *S. dublin*. On May 6, 1946, approximately four months after the affected animal was removed from the herd, blood and fæces samples were taken from the remaining cattle and examined. One animal with a normal serum titre was found to be excreting *S. dublin*.

Result of periodical exa

No. of animal	24-4-46 "H"	24-4-46 "O"	24-4-46 Fæces	20-5-46 "H"	20-5-46 "O"	20-5-46 Fæces	15-7-46 "H"	15-7-46 "O"	15-7-46 Fæ
80	1/10	1/40	Neg.	1/10	1/10	Neg.	1/10	1/10	S
81	1/2560	1/1280	*S.d.*	1/1280	1/1280	*S.d.*	1/2560	1/640	S
74	Neg.	1/20	Neg.	1/10	1/20	*S.d.*	1/10	Neg.	N
78	1/2560	1/1280	*S.d.*	1/1280	1/2560	*S.d.*	1/2560	1/640	S
76	1/20	1/80	*S.d.*	1/40	1/80	Neg.	1/40	1/40	N
71	1/10	1/40	Neg.	1/10	1/20	Neg.	1/10	1/20	N
Calf of No. 74 White heifer							1/10	Neg.	S
Grey cow							NS	NS	S

NS = Not samp

CASE NO. 6

Result of the examination of a herd four months after a clinical case of salmonellosis

No. of animal	Fæces	Blood titre "H"	Blood titre "O"	No. of animal	Fæces	Blood titre "H"	Blood titre "O"
1	Neg.	Neg.	1/10	7	Neg.	1/10	1/40
2	Neg.	1/80	1/40	8	Neg.	1/10	1/80
3	Neg.	1/20	1/40	9	*S. dublin*	1/10	1/40
4	Neg.	Neg.	1/20	10	Neg.	1/40	1/40
5	Neg.	Neg.	1/10	11	Neg.	1/10	1/40
6	Neg.	Neg.	1/10	12	Neg.	Neg.	1/40

At the same visit blood and fæces samples were taken from a young calf. The fæces were negative for *S. dublin* but the blood showed flagellar agglutinins to this organism to a high titre, indicating that the calf had been exposed to infection with this organism.

This finding, together with the isolation of the organism from the fæces of animal No. 9 at this test, indicates that even with the elimination of the animal recovered from infection a focus of infection remained on the farm.

The Laboratory Diagnosis of Salmonellosis

(a) BACTERIOLOGICAL.—A diagnosis of salmonellosis based on clinical symptoms cannot be made with certainty. At least three outbreaks of disease in herds of cattle have been investigated during the period under review, having clinical syndromes suggestive of salmonellosis but which were clearly shown not to be associated with salmonella infection, while cases proved to be salmonellosis by laboratory diagnosis have on occasion been incorrectly diagnosed by veterinary surgeons in the field. The point is stressed, therefore, that while the clinical picture

n of animals in herd

21-10-46			21-4-47			14-7-47			24-9-47		
"H"	"O"	Fæces	"H"	"O"	Fæces	"H"	"O"	Fæces	"H"	"O"	Fæces
			1/20	1/10	Neg.	1/20	Neg.	Neg.	1/40	1/80	Neg.
			1/1280	1/160	*S.d.*	1/1280	1/160	*S.d.*	1/640	1/160	*S.d.*
			1/10	Neg.	Neg.	Neg.	Neg.	Neg.	NS	NS	NS
2560	1/1280	*S.d.*	1/1280	1/160	*S.d.*	NS	NS	NS	1/1280	1/320	*S.d.*
	Sold										
S	NS	NS	1/20	Neg.	*S.d.*	1/20	Neg.	Neg.	1/40	1/40	*S.d.*
			1/1280	1/20	*S.d.*						

Neg. = Negative. *S.d.* = *S. dublin.*

may give strong presumptive evidence of salmonella infection, diagnosis must be confirmed by the demonstration of the specific organism in the blood and/or fæces of the affected animals.

Media.—Trials were made with various media in order to determine the most satisfactory method of isolating salmonella organisms under the conditions prevailing in a routine diagnostic laboratory. MacConkey's bile salt neutral red agar. Wilson & Blair's medium and desoxycholate citrate agar were used as solid selective media. Tetrathionate broth and selenite broth were used as liquid enrichment media. No critical comparisons were made of the relative value of the different media, each being judged on the results obtained by its employment. Of the solid media, neither MacConkey's agar nor Wilson & Blair's medium gave results comparable to desoxycholate citrate agar. This medium has the additional advantages of being easily prepared and remaining stable in the laboratory for long periods of time. Of the liquid enrichment media selenite broth was found preferable to tetrathionate broth. These two media have been used during the grater part of this investigation. D.C.A. has been prepared by Hynes' modification of Leifson's method (Mackie and McCartney, 1942) except that the solutions A and B have been dissolved in the waterbath for 18 hours at 53 deg. C. and no further sterilisation has been carried out. S.B. has been prepared by the method described by Hobbs and Allison (1945).

Technique.—The following techniques have been found satisfactory for the isolation of salmonella organisms from the different specimens. In all cases, unless otherwise stated, cultures were incubated for 18-24 hours at 37 deg. C.

Fæces samples.—A large loopful was plated directly on to a D.C.A. plate and a similar loopful seeded into 15 c.c. S.B. Following incubation the D.C.A. plates were examined. If the D.C.A. plates were negative, a sub-culture was made from the S.B. to D.C.A. The resulting cultures were examined and any non-lactose fermenting colonies tested with salmonella agglutinating sera.

Blood samples.—After centrifugalisation of the sample, the serum was removed and tested for the presence of agglutinins to *S. dublin*. The clot was placed in a sterile petri dish and broken up with sterile forceps. Small pieces were then seeded into 15 c.c. of S.B. After incubation a sub-culture was made on to D.C.A. and the resulting growth examined for salmonella organisms.

Milk samples.—Ten c.c. of milk were seeded into 15 c.c. S.B. After incubation a sub-culture was made on to D.C.A. and the resulting growth examined after incubation.

Results.—The results presented refer to 63 positive clinical cases of salmonella infection. The affected animals were of all ages from young calves to lactating cows.

Fæces.—Fæces samples were submitted from 62 of the 63 cases. In the remaining case, a blood sample only was obtained. Salmonella organisms were recovered from 61 of the 62 fæces samples. The sample proving negative was taken from an animal during the febrile stage of the disease and before the development of enteric symptoms. The organism was, however, recovered from the blood.

Blood.—Blood samples were submitted from 26 of the 63 positive cases. A number of these were forwarded before a satisfactory technique had been devised for the isolation of organisms from the blood clot. The results refer, therefore, to 15 of these samples which were received after the satisfactory method of isolation had been devised. Of the 15 samples, nine yielded salmonella while six samples were negative.

Milk.—Only five samples were submitted from the 63 positive cases: four yielded salmonella and one was negative.

Identification of organisms.—A complete examination of the strains isolated has not been undertaken by the writer. Examination has been confined to the determination of certain biochemical characteristics and the placing of the organism in its group by the use of group sera. Complete examination of the strains by antigenic analysis has been carried out by Mr. A. Buxton, of the Veterinary Laboratory, Ministry of Agriculture and Fisheries.

The almost complete agreement as to the identity of the strains submitted to him for examination suggests that the technique adopted by the writer is adequate for the ordinary diagnostic laboratory.

Isolated non-lactose fermenting organisms were picked off from D.C.A. plates and emulsified in normal saline. Slide agglutination tests were carried out using polyvalent salmonella serum and the group sera B.C.D. and E. Strains agglutinating with the polyvalent serum and with one of the group sera were sub-cultured on two nutrient agar slopes and in peptone water. Sub-cultures were made from the peptone water after six hours' incubation at 37 deg. C. into lactose, sucrose, salicin, glucose, maltose and mannite. If the organism was agglutinated by polyvalent salmonella serum, produced no change in lactose, sucrose and salicin but produced acid and gas or acid only in glucose, maltose and mannite, it was accepted as a salmonella. When such organisms were agglutinated by group B sera they were tentatively regarded as *S. typhi-murium* and when agglutinated by group D

sera as *S. dublin*. Agar slope cultures of such organisms were submitted to the Veterinary Laboratory, Weybridge, for confirmation.

Variants of S. dublin.—A number of variant strains of *S. dublin* has been isolated during the present survey. The examination of these strains forms the subject of a separate study and it is sufficient to mention here that variants isolated have included non-gas producing (anærogenic), mucoid and non-motile strains, while many strains show variation in biochemical characteristics.

(b) SEROLOGICAL.—Organisms of the salmonella group are all motile with the exception of *S. gallinarum*. They therefore possess both flagellar and somatic antigens and stimulate the production of flagellar and somatic agglutinins. Lovell (1934) showed that the sera of cattle contained both "H" and "O" agglutinins to various species of salmonella.

In view of this finding and before any diagnostic significance could be attached to an agglutination test, it was considered necessary to determine the normal range of agglutinins in bovine sera to *S. dublin*. This was done in order to have a basis upon which to assess the significance of blood titres at various stages of illness and during the carrier state. In view of the amount of technical work involved, the observations have been confined to the reaction to *S. dublin* antigen.

Technique

Preparation of antigens.—The greater part of the antigen used in this work was prepared by Mr. A. Buxton, M.R.C.V.S., of the Veterinary Laboratory, Ministry of Agriculture and Fisheries. A small amount has been prepared by the writer using the methods adopted at the Weybridge laboratory.

(a) *Flagellar antigen.*—A selected fully motile strain of *S. dublin* was seeded into 1000 c.c. of peptone broth and incubated at 37 deg. C. for 24 hours. Ten c.c. of 40 per cent. formalin were then added gradually, the flask being shaken vigorously. After standing for at least one hour the antigen was tested against a *S. dublin* serum of known titre.

(b) *Somatic antigen.*—A selected strain of *S. dublin* was sown into nutrient broth and incubated at 37 deg. C. for 18 hours. Roux flasks containing nutrient agar were inoculated with approximately 5 c.c. of the broth culture and incubated at 37 deg. C. for 48 hours. The resultant growth was washed off with sterile normal saline and sterile glass beads. Washings were filtered through glass wool and collected in one flask. To the washings was then added absolute alcohol (170 c.c. absolute alcohol to 100 c.c. washings). The mixture was shaken and allowed to stand for 48 hours when it was centrifugalised and the supernatant alcohol and saline drawn off. The deposit was re-suspended in normal saline and standardised to a density equal to Brown's opacity tube No. 2. The antigen was tested against a *S. dublin* "O" serum of known titre.

Preparation of agglutination tests.—Agglutination tests were prepared by the normal method of making dilutions of sera in geometrical progression. The resulting suspensions were incubated in a waterbath at 53 deg. C. Readings of "H" agglutination were made after two hours' and again after 18 hours' incubation. A reading of "O" agglutination was made after 18 hours' incubation.

Results

The serum level of normal bovine animals.—A herd of cattle was selected for test in which careful enquiry had failed to show any evidence of previous salmonella infection. Blood samples were taken from the ill animals in the herd and subjected to an agglutination test. The ages of all the animals were obtained in order, if possible, to correlate the level of agglutinins in the blood with various age groups. The results are shown in Table 5. In this table the animals have been divided into age groups, in order to illustrate a relationship which apparently does exist between the level of agglutinins and length of life. Of the 28 animals less than 12 months of age, 26 had no flagellar agglutinins or reacted only at a titre of 1/10. One of the remaining animals had a titre of 1/20, and the other, one of 1/80. An almost parallel result was obtained with somatic antigen, 26 young animals having no somatic agglutinins or reacting only at a titre of 1/10, while of the two remaining animals, one had a titre of 1/20 and the other a titre of 1/40.

By comparison, a consideration of the 41 animals in the age group 1-3 years shows that while 17 animals had no flagellar agglutinins or reacted only at a titre of 1/10, 23 had titres in the range of 1/20 to 1/80, while one showed a titre of 1/160. Similarly, while 19 animals had no somatic agglutinins or reacted only at a titre of 1/10, 20 had titres of 1/20 and two a titre of 1/40. It is clear, therefore, that the level of agglutinins of animals in this age group was significantly higher than in animals in the younger age group. Reference to the table indicates also that the agglutinating titres of animals in the two age groups, 3-5 years and 5-8½ years, were approximately the same as those of animals in the age group 1-3 years. Assuming these results to be representative of the reactions of a normal herd of cattle, then the majority of normal healthy animals under one year of age either have no serum agglutinins to *S. dublin* or, where present, they are quantitatively weak. Agglutinins to a higher titre appear in the blood between the first and third years of life. The highest flagellar titre found in this herd was 1/160 and the highest somatic titre 1/40.

(To be continued)

THE DEVELOPMENT OF THE STOMACH IN THE RUMINANT

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Introduction

THE mode of development of the fore stomachs of the ruminant has been a matter of conjecture for some time. In many textbooks one reads statements to the effect that the ruminant abomasum corresponds to the simple stomach of, say, man or the horse, and that the rumen, reticulum and omasum develop later

as dilatations of the œsophagus, these dilatations being lined with the same stratified squamous epithelium as is found in the œsophagus.

The opinions of several workers have been based on their study of the development of the epithelial cells lining the œsophagus or stomach. Zimmerman and Sal (1894) claimed to have discovered cardiac glands along the œsophageal groove in the sheep. They drew attention to the fact that only fundic and pyloric glands were found in the stomach of reptiles and batrachians and suggested that cardiac glands were œsophageal glands appropriated or absorbed into the region of the stomach.

Oppel (1896) proposed that the fundic and pyloric types of gastric glands were differentiated forms of the simple glands of the lower vertebrates and that cardiac glands were decadent or retrogressive fundic glands.

Thus, it might appear that cardiac glands are appropriated œsophageal glands, that they are modified fundic glands, or that they are glands which have developed in response to a new functional demand.

Bensley (1902), when discussing those Orders which presented so-called œsophageal dilatations, said: "Moreover, in the Ungulates, no existing member of which exhibits a perfectly simple stomach, the nearest approach to this is found in the animals (the pigs in the Artiodactyla and the tapirs in the Perissodactyla) which have been conservative in other respects, for example, in dentition or in foot structure. . . . In all other mammals, the evidence is that the complex stomachs, such as those of the ruminants and rodents, etc., are produced from a simple stomach by progressive specialisation, in which the œsophagus takes no part, and in which the important phenomena are the suppression of the gastric glands and the replacement of the mucigenous epithelium by a stratified squamous epithelium."

Heuser is quoted by Lewis (1915) as saying: ". . . the fundus in the sheep develops into the large rumen. Even though it is lined with stratified squamous epithelium, as in the adult, it cannot be regarded as a portion of the œsophagus as some have taught."

Pernkopf (1931) has published an excellent and detailed account of the development of the ruminant stomach, particularly in the early stages. He also maintains that the œsophagus takes no part in the formation of the compound stomach. He states that the lining of the stomach is in no way specific and that it is in no way associated with a definite form of stomach or with definite divisions. On the contrary, he points out that even in the simple stomach of Monotremata (e.g., the duck-mole or echidna), it can be demonstrated that the epithelial lining of the stomach is one of keratinised stratified squamous epithelium.

Material and Methods

The specimens throughout were bovine embryos or fœtuses. The embryos were measured when fresh, before shrinkage had taken place in formalin. The measurements were from the crown to the rump. The sections which were prepared from the embryos were embedded in paraffin wax. Staining was by hæmatoxylin and eosin.

The reconstructed model was made from paraffin wax (M.P. 52° C.) with an

admixture of resin to harden, in the proportion of one part of resin to ten parts of paraffin wax.

Terminology

The term " stomach " has been used to include rumen, reticulum, omasum and abomasum in the study of these compartments in the ox. If Dukes (1942) be correct, this would not apply to all ruminants, as he says that some, in the Tragulidæ and Moschidæ, do not possess an omasum.

Early Development of the Stomach

In no way does the early development of the stomach differ from that of any other mammal. The first sign is a long ovoid dilatation, clearly seen in an embryo of approximately 7 mm. This is represented in Fig. 1. It is simple. It is the same, in all respects, as the early dilatation of the primitive gut in other animals. It is from this dilatation that the rumen, reticulum, omasum and abomasum develop. There is no constriction separating the œsophagus and stomach, and no constrictions appear in the œsophagus to differentiate the areas of the future rumen, reticulum and omasum. The fact is, that the compartments differentiate concurrently.

In ox embryos of 9 mm. length, the stomach shows externally an area which delineates the future position of the œsophageal groove. In Fig. 1, at E, in the model of a stomach of a 9 mm. bovine, it is seen that the rumen becomes more prominent than the other compartments. D shows the area of the developing œsophageal groove. It is to be noted that the groove lies along the concave lesser curvature. Its floor is the line of the stomach wall along that lesser curvature. An arrow, passing from the outside and passing between the lips of the groove, and equi-distant from them, would transfix the line of the greater curvature of the stomach in some part of its length.

The model of the stomach taken from an embryo of 14 mm. in size (Fig. 1) shows the rumen to be still more prominent. At this stage of development, an indefinite area can be apportioned to the omasum at F, and to the abomasum at G.

Thus far, it may be seen that the rumen is a compartment which develops dorsally and anteriorly. The omasum is a compartment which develops ventrally and posteriorly. The abomasum is a compartment which develops dorsally and posteriorly. The reticulum has only hinted its appearance, but is destined to become apparent on the left side of the stomach, between the œsophagus and the omasum.

The Stomach in an Embryo of 30 mm. Length

Reference to a model (Figs. 2 and 3), sectioned in four parts and reconstructed in wax from serial sections of the stomach of a 30 mm. ox embryo, will assist in demonstrating the growth and development of the rumen, reticulum, omasum and abomasum.

The rumen is seen to be an almost cylindrical structure, lying dorsal to the œsophagus and being the most anterior of the four compartments of the stomach. The reticulum lies to the left and slightly dorsally. It is between the rumen

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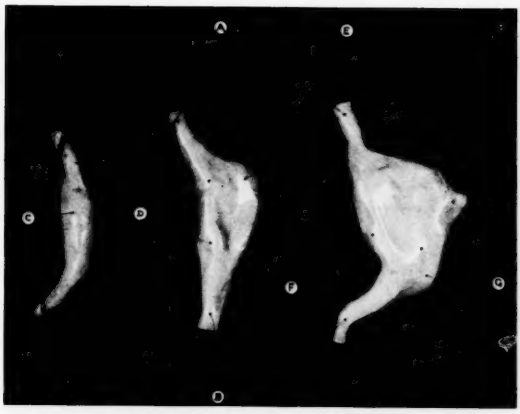

FIGURE 1.—Bovine stomach, left side. (Adapted from Pernkopf.)
Left to right: Models of stomachs from 7, 9 and 14 mm. embryos.

A—œsophagus.
B—area of pylorus.
C—fundus of stomach.
D—area of œsophageal groove.
E—developing rumen.
F—developing omasum.
G—developing abomasum.

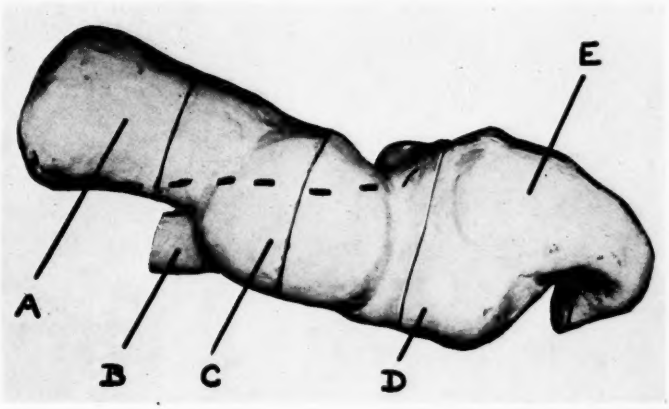

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FIGURE 2.—Model in wax, of stomach from 30 mm. ox embryo.
A view from the left and dorsally.

A—rumen.
B—œsophagus.
C—reticulum.
D—omasum.
E—abomasum.
Dotted line—attachment of dorsal mesentery.

Article by P. S. Lambert]

XUM

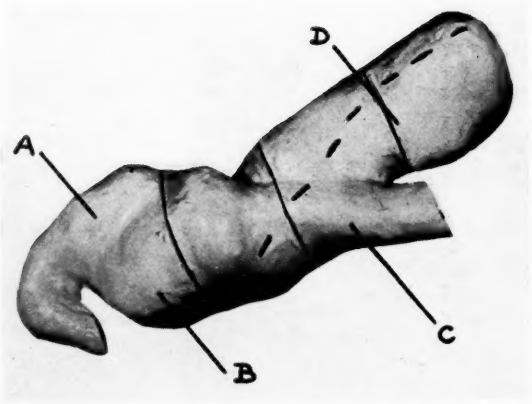

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FIGURE 3.—Model in wax from a stomach of a 30 mm. ox embryo. Viewed from the right and ventrally.

A—abomasum.
B—omasum.
C—œsophagus.
D—rumen.
Dotted line—attachment of dorsal mesentery to rumen and ventral mesentery to omasum and abomasum.

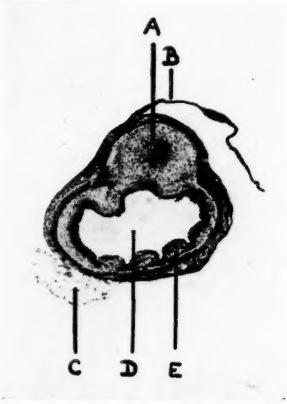

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FIGURE 4.—Section from 30 mm. ox embryo.

A—wall of abomasum.
B—attachment of dorsal mesentery.
C—connective tissue, concealing torn ventral mesentery.
D—cavity of omasum.
E—omasal leaf.

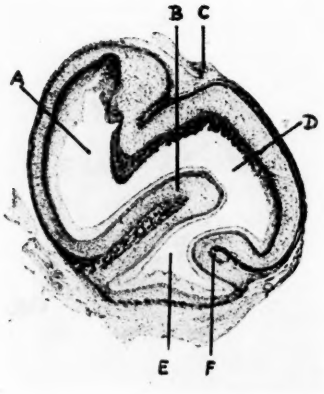

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FIGURE 5.—Section from 55 mm. ox embryo.

A—cavity of rumen.
B—right lip of œsophageal groove.
C—attachment of dorsal mesentery.
D—cavity of reticulum.
E—lumen of œsophageal groove.
F—left lip of œsophageal groove.

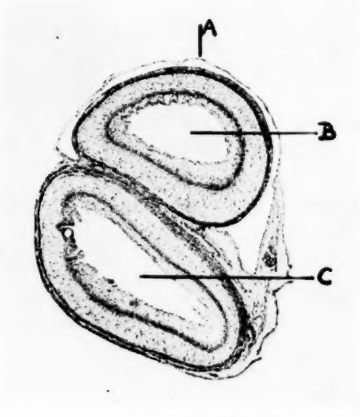

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FIGURE 6.—Section from a 50 mm. ox embryo.

A—pouch from dorsal mesentery.
B—future ventral blind sac (adult animal).
C—future dorsal blind sac (adult animal).

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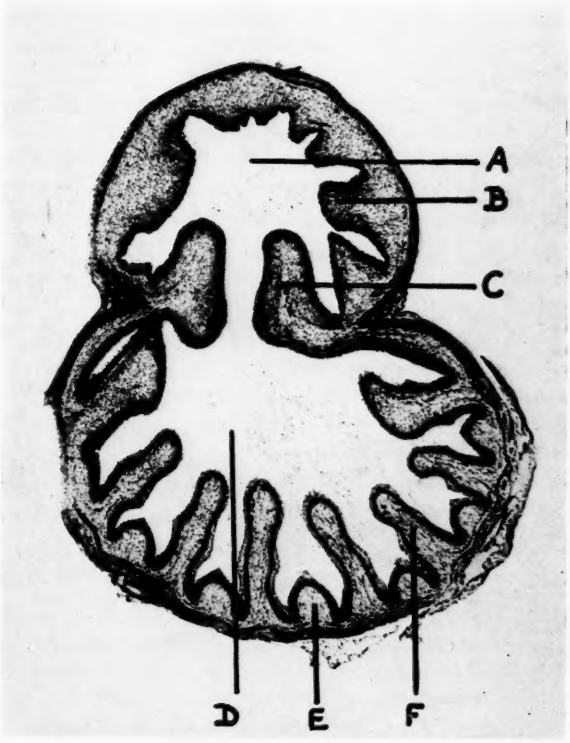

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FIGURE 7.—Section from 50 mm. ox embryo.

A—cavity of abomasum.
B—spiral fold.
C—omaso-abomasal valve.
D—cavity of omasum.
E—secondary omasal leaf.
F—primary omasal leaf.

Article by P. S. Lambert]

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(a) Diagram of 30 mm. ox embryo. Dorsal view. Shading shows dorsal and part of ventral mesentery.

(b) Left and (c) right sides of stomach, from a 25 cm. ox fœtus.

(d) Stomach from a 25 cm. ox fœtus. Connective tissue adhesions between compartments have been broken down.

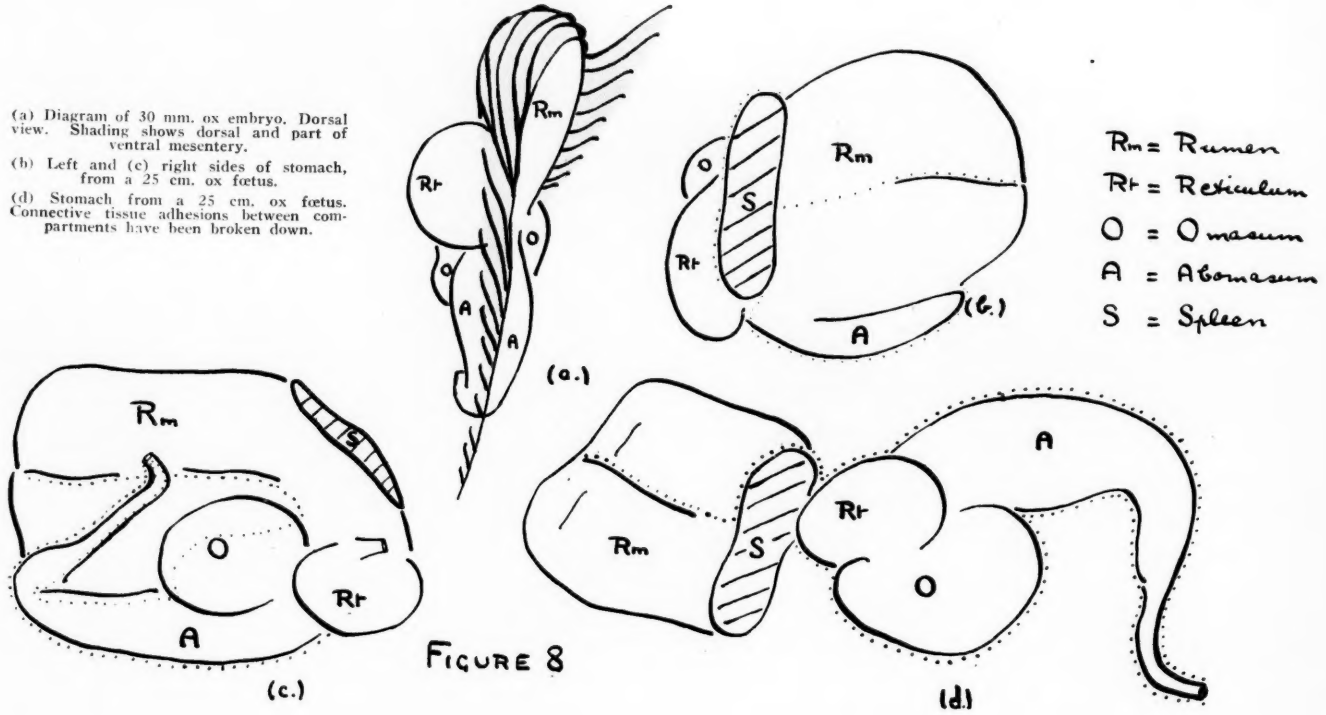

FIGURE 8

and the omasum and has an appearance from the outside of an hemispherical bulge. Posteriorly and ventrally, lies the omasum. At this stage, the omasum is broader than any of the other compartments. Dorsally to the omasum and running posteriorly is the abomasum, and this is somewhat flask-shaped, the neck merging into the duodenum. There is no definite indication of the pylorus at this stage.

The various compartments of the stomach become more apparent when the interior of the stomach is examined. Entering the stomach at what may rationally be termed the cardia, then turning dorsally and anteriorly, one enters the rumen. At this stage of development, the rumen is very thick-walled. There is no folding of the epithelium to form pillars of any sort. The epithelium is exactly similar to that of the œsophagus, being typical primitive stratified squamous epithelium, the cells of the surface-layer being large, full and polygonal, with spheroidal nuclei and not showing the flattened squames or the keratinisation which is seen in the adult. There are, as yet, no papillæ to be seen.

Returning to the cardia and proceeding posteriorly, one observes, on either side, two low walls, both of equal size, formed of folds covered by stratified squamous epithelium. These are the developing lips of the œsophageal groove. Beyond that wall, which is the embryo's left, lies the reticulum. Here again the epithelium is typically primitive stratified squamous epithelium, there being no flattening of the surface cells, although there are about a dozen layers. There is no suggestion of the formation of the characteristic honeycomb of the adult reticulum. Proceeding posteriorly, the œsophageal groove is seen to end abruptly and four sagittally disposed ridges lie stretched beyond. These are the first of the numerous omasal leaves of the adult.

Fig. 4, which is a section cut from a 30 mm. embryo, will show the extent to which already the leaves are developed in the early omasum.

In the region of the roof of the omasum lie two parallel, low, sagittal folds of epithelium, projecting downwards. These bound the future omasal sulcus of the adult and run the length of the roof of the omasum, as do the omasal leaves with respect to the floor. Posteriorly, this groove is seen to end suddenly by dipping into the omaso-abomasal opening, and the folds bounding the groove meet each other posteriorly, to form the valve-like omasal pillars. As in the case of the rumen and the reticulum, the epithelial lining of the wall of the omasum, its leaves and sulcus, is one of early stratified squamous epithelium.

It is seen, then, by referring to the three models in Fig. 1, that the omasal sulcus develops from a length of the convex greater curvature of the stomach. Passing through the omaso-abomasal opening, at the posterior part of the roof of the omasum, the lining of the abomasum is found to possess several folds, running in directions similar to that of the spiral folds in the adult. The epithelium shows layers of cells which are more columnar than in the rumen, reticulum and omasum, but there is as yet no differentiation into gastric glands, let alone into glands of cardiac, fundic and pyloric types. The nuclei are larger and more ovoid than in the cells lining the previous three compartments.

The Stomach in an Embryo of 50 mm.

At this stage the relations of the various compartments are not greatly altered. Each compartment, however, has pouched out more, thus making the boundaries of each part quite definite. This period of development is especially notable for two points:—

Firstly, the early sections from serial cutting showed that the rumen, anteriorly, had been divided into a dorsal and ventral sac by a folding of the epithelium. (See Fig. 6.) As will be described later, this transverse folding of epithelium, lying at the anterior extremity of the rumen, is destined to become the posterior transverse ruminal pillar of the adult. Two sacs lie above and below the groove at this extremity, becoming the ventral and dorsal posterior blind sacs of the adult, respectively.

Secondly, sections of a 50 mm. ox embryo prove interesting with regard to the œsophageal groove. Fig. 5 illustrates the precocious development of the lips of the œsophageal groove. The exceptional feature is the very great disparity in the size of the lips at this stage, and it is remarkable that the twist to the œsophageal groove in the adult, which is formed by a large lip turning around a smaller, rather than by the intertwining of lips of equal size, should be foreshadowed so early in development by the greater growth of one lip. This large lip is on the right at this stage. By the change in the position of the rumen, described later, this large lip remains on the right at the œsophageal opening only, and in relation to the left lip is to the right, posterior, and then to the left in its course from the œsophagus to the reticulo-omasal opening. Hence, the lips of the groove point backwards, then to the left, and finally forwards.

With regard to the interior of the stomach at this stage of development, the ruminal epithelium presents an appearance of typical stratified squamous epithelium without cornification. The layers of epithelial cells exceed twenty throughout the rumen and columns of cells project into the lumen in some places. (Fig. 6.)

The reticulum has as many layers, but projections of epithelium into the lumen have definite square-cut corners, which indicate the early boundaries of the future honeycomb appearance. (Fig. 5.)

The omasum shows long primary leaves and small secondary leaves. There is an epithelial covering of stratified epithelium; but here the layers are considerably less, being about four in number except at the tips of the leaves and between the leaves, where the layers number about ten. There are very pronounced valve-like omasal pillars at the omaso-abomasal opening. (Fig. 7.)

The abomasum shows a layered epithelium, but the cells are quite different from those of the reticulum, rumen and omasum, being definitely columnar, closely packed and with large ovoid nuclei, and the luminal surface of the epithelium being smooth and even. There is, as yet, no differentiation into the various types of gastric gland.

The Formation of the Omenta

The greater and lesser omenta in the ox have complicated attachments and foldings. If these attachments and foldings are followed in development, then

much simplification ensues in understanding the arrangement in the adult.

As in all developing stomachs, the early spindle is attached by a dorsal mesentery and a ventral mesentery. They are double-layered, each layer parting to pass round its respective side of the stomach spindle and meeting again ventrally. The dorsal mesentery is due to form the greater omentum and the ventral to form the lesser omentum. The dorsal mesentery is attached to the convex or greater curvature of the developing stomach. The ventral mesentery is attached to the concave or lesser curvature of the developing stomach. (See Fig. 1.)

The rumen pushes forward, dorsal to the œsophagus. As it does this, it rotates to the left and the original anterior part of the dorsal curvature of the developing stomach-spindle passes to the left and carries the dorsal mesentery with it. The point of attachment is along the future left longitudinal groove of the rumen. Similarly, the ventral mesentery is pulled to the right and is attached along the future right longitudinal groove of the rumen. (See Figs. 2, 3 and 8a.)

If the lines of attachment of the dorsal and ventral mesenteries are followed, then it will be seen that the dorsal is attached along the future left longitudinal groove, then over the reticulum, then over the greater curvature of the abomasum, which is still dorsal in the stomach of a 30 mm. long embryo. (Figs. 2, 3 and 8a.)

Again, if the line of attachment of the ventral mesentery is followed, it is seen to pass along the future right longitudinal groove, then ventrally, to the anterior part of the omasum. From this point, it is attached in the mid-line ventrally, to the omasum and to the lesser curvature of the abomasum.

Fig. 8b is a drawing of the left side of a stomach from a 25 cm. fœtus. The stomach at this stage is very similar in disposition and relative size of its compartments to that of the adult, although variations in the sizes of the compartments occur later in the life of the fœtus and till about 18 months old in the young ox. (Sisson, 1940.) The reflections of the peritoneum are almost the same as in the adult animal, so that stomachs from a fœtus of this size afford easily handled material for a study of the disposition of the peritoneum.

In Fig. 8b, the anterior dotted line (vertical) indicates the course of the reflection of the visceral peritoneum from the spleen to the abdominal wall and diaphragm. The two dotted horizontal lines show the reflections of the peritoneum from the left longitudinal groove and the convex greater curvature of the abomasum. These lines of reflection are continuous beneath the ventral extremity of the spleen and involve a small part of the wall of the reticulum. In Fig. 8d, the attachments of the compartments by connective tissue have been broken down and an arrangement of the compartments exactly similar to that obtaining in the 30 mm. embryo has been achieved. Here the line of reflection may be clearly followed along the left longitudinal groove, the ventral extremity of the spleen, the reticulum, and the greater curvature of the abomasum. Now let this be compared with the line of reflection of the mesentery in a 30 mm. embryo. (See Fig. 2.) Except for the developing spleen—not present on the model—the structures involved are identical.

The dorsal mesentery, therefore, forms what Sisson calls the superficial layer of the greater omentum. Now, the reflection of the peritoneum from the right longitudinal groove to the liver and pancreas is described by Sisson as the deep part of the greater omentum. The right longitudinal groove, however, as described previously, has the ventral mesentery attached and resulted from the rotation of the rumen so that the original attachment of the mesentery to the mid-line of the early stomach-spindle was pulled, as far as the anterior part of the spindle is concerned, to the right. Hence, what Sisson describes as the deep part of the greater omentum is, in fact, a part of the ventral mesentery and therefore a part of the lesser omentum.

Continuing the attachment of the ventral mesentery posteriorly, it is seen to be attached to the parietal face of the omasum and to the lesser curvature of the abomasum. (Fig. 8d, and compare Fig. 3.) The peritoneum from these compartments passes to the liver. This is the lesser omentum of Sisson.

The Twist of the Œsophageal Groove

The breaking down of the connective tissue adhesions of the compartments to one another and their arrangement as in Fig. 8d involves no strain or twisting in any part of the wall of the stomach. The compartments fall easily into relations which are the same as in an embryo of 30 mm. If, now, the reticulum be opened and the floor of the œsophageal groove be placed ventral, as in the developing stomach, then the lips of the œsophageal groove are parallel and sagittal and project dorsally. There is, in fact, no longer any twist to the œsophageal groove.

The Change of Position of the Compartments in Development

The rumen may be said to revolve about a longitudinal axis before the embryo is 30 mm. in size, and it revolves about a transverse axis at the point of the entrance of the œsophagus. Eventually it rotates about this transverse axis through 180 deg. When the embryo is 50 mm. in length, it has completed almost half of this movement, and the long axis of the rumen is, at this stage, pointing dorsally and slightly forwards. This change is probably assisted by the developing liver pulling the rest of the developing stomach ventrally and by lack of corresponding growth of the dorsal mesentery, and by the fact that there is not room to accommodate the energetically growing rumen anteriorly. Whatever the causes, what was the anterior dorsal blind sac becomes the posterior ventral blind sac, and what was the anterior ventral blind sac becomes the dorsal posterior blind sac. Hence, the left longitudinal groove of the rumen, where the dorsal mesentery is attached, is approximated to the greater curvature of the abomasum.

The reticulum, omasum and abomasum all revolve through approximately 180 deg. (See Fig. 8b, c and d and compare with Fig. 1.) The parietal face of the omasum faces to the right and dorsally, although originally ventral, and the greater curvature of the abomasum becomes ventral, originally dorsal.

The points of attachment and reflection of the peritoneum are retained:—

1. From the right longitudinal groove to the liver and pancreas, developing ventrally. This is part of the lesser omentum. (The deep part of the greater omentum of Sisson.)

2. From the parietal face of the omasum and the lesser curvature of the abomasum to the liver, developing ventrally. This is the lesser omentum (Sisson's). Actually, it is only a part of the ventral mesentery from the stomach, the rest being the so-called deep part of the greater omentum. (See Figs. 3, 4 and 8.)

3. From the left longitudinal groove to the ventral extremity of the spleen, to the reticulum and to the abomasum. This is the greater omentum. (See Figs. 3 and 8.) It is the superficial part of the greater omentum of Sisson.

Conclusions

Arey (1946) describes the convex greater curvature of the human stomach, primarily dorsal in the embryo, as being produced by greater growth of the dorsal border of the stomach, and thus passively resulting in the concave lesser curvature. Broman (1911) describes how the fundus of the stomach develops as a local bulge near the cranial extremity of the stomach, and on the dorsal border, and how in the third month of development in the human embryo this outpocketing is directed so markedly cranial that its blind end comes to lie above the orifice of the œsophagus. This bulge in the human embryo becomes the fundus of the adult stomach. In the ox, this bulge becomes the rumen. On anatomical grounds, then, the rumen may be said to correspond to the fundus of the human stomach. (Cf. C. H. Heuser, quoted above.)

Toldt (1879) says that in the human embryo the œsophagus enters the stomach in such a way that the lesser curvature forms, as it were, a continuation of the ventral border of the œsophagus. Lewis (1912) concludes that the œsophagus, in joining the stomach, forms a cone which reaches as far as the angular incisure. This cone gives rise to the cardiac antrum, cranially, and a prolongation caudally, extending along the lesser curvature. This is the gastric canal. Lewis also says that in the same way as the gastric canal accords with the œsophageal sulcus in ruminants, then the cardiac antrum may correspond to the atrium ventriculi, a dome-shaped swelling on the dorsal side of the reticulum and at the thoracic end of the rumen. He further maintains the œsophageal sulcus to be described by comparative anatomists as a continuation of the œsophagus, open on one side. He does not, however, continue the argument to its conclusion, for the comparative anatomists had maintained that the rumen, reticulum and omasum were œsophageal derivatives, in which case the fundus of the human stomach, the corpus, and no little part of the pyloric area, would have to be considered as really part of the œsophagus! It is much more reasonable to regard the œsophageal sulcus as corresponding to the gastric canal and that the œsophageal groove should be termed the gastric canal, for there is no entrance of the œsophagus into the stomach-spindle in the shape of a cone, in the case of the ox embryo. The œsophageal sulcus is truly part of the early stomach-spindle. (See Fig. 1.) The rumen and the fundus of the stomach are the comparable. The left side of the corpus of the human stomach is comparable to the reticulum. The omasum corresponds to the lesser curvature of the human stomach, caudal to the point where the gastric canal stops. The abomasum is comparable to the pyloric area of the human stomach. Epithelial linings are secondary characters.

Summary

1. The rumen, reticulum, omasum and abomasum all develop from the same spindle-shaped dilatation.

2. These compartments assume epithelial linings which are distinctive in an ox embryo of size 30 mm.

3. The epithelial linings of any particular compartment afford no basis for deducing the manner in which that compartment develops. The œsophagus may be said to end in the embryo where the stomach-spindle begins, and no compartment whatever originates as a pouch from the œsophagus. The œsophageal groove would be better termed the gastric canal.

4. The formation of the greater and lesser omenta is comparable to their formation in any other animal with a simple stomach, where the foldings are less complicated.

Acknowledgments

My thanks are given to Mr. H. V. Hughes, B.V.Sc., M.R.C.V.S., D.V.H., and to Mr. J. W. Dransfield, M.V.Sc., M.R.C.V.S., for their help and advice and to the latter for his able German translations. I also thank Mr. T. Fleming, Technician in this Department, for untiring help and excellent photography.

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LISTERIA MONOCYTOGENES IN VOLES

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LISTERIASIS has been reported in man and in a large number of domesticated species such as guinea-pigs, rabbits, sheep, cattle, goats, swine, fowls, a foal, a dog, and also in foxes bred in captivity. In wild animals the disease was recorded in gerbilles in South Africa, in hares and a capercaillie in Sweden, and in a rat

in Brazil. This note deals with the isolation of *Listeria monocytogenes* from voles kept under laboratory conditions.

Losses occurred among 39 voles (Microtus agrestis) kept in two separate groups. These animals were one-five months old and were under observation after experimental inoculation with different species of Mycobacteria. In the ensuing six months, 24 voles died of intercurrent diseases, and seven of these were infected with Listeria. The seven voles were found dead at different times, no premonitory symptoms being noticed. Post-mortem examination showed very numerous necrotic foci, 0.1-2 mm. in diameter, in the liver and spleen. These organs were enlarged, especially the spleen, which extended in some cases half-way to the pelvis. The intestines were congested, their contents sometimes being streaked with blood. Pin point necrotic foci were present in the mesenteric nodes of some animals. The lungs and kidneys appeared normal.

Smears of the focal lesions showed a fair number of gram-positive rods situated inside or outside leucocytes. These organisms could be isolated from the blood and the internal organs. On nutrient agar plates, growth occurred in 24 hours at 37° C. in the form of circular, smooth, slightly convex translucent colonies about 0.6 mm. in diameter with an even edge. They grew better on 5 per cent. bovine blood agar, the colonies being larger, sometimes confluent and surrounded with a narrow zone of more or less complete hæmolysis. A uniform slight turbidity occurred in broth with very little deposit, and sometimes a very thin pellicle. Motility was noted at 37° C. as well as at room temperature. Gelatin and solid serum were not liquefied. On MacConkey agar the colonies were isolated and pink in colour. Sugar fermentation was studied in peptone water medium in presence of Andrade's indicator. Acid was produced from glucose, maltose and salicin. Sucrose and mannitol were not attacked. Lactose, as a rule, was not fermented, or only very slightly, except by one strain which produced a fair amount of acid. Litmus milk was acidified. Further tests gave the following results :—

Indol negative, M.R. positive, V.P. negative; nitrate reduction negative; NH_3 production in peptone water negative, citrate utilisation negative, methylene blue reduction positive, H_2S production negative, catalase positive.

These characteristics were similar to those of a strain of *Listeria monocytogenes* isolated from a lamb.

Resistance

The survival of four vole strains was studied. These were grown on nutrient slopes such as agar (*pH* 7.2-7.4), 5 per cent. bovine blood-agar, inspissated horse serum and egg for 24 hours at 37° C., then covered with liquid paraffin and kept at room temperature. Under these conditions the organism survived for at least three years. Cultures dried in vacuo over phosphorus pentoxide and kept at room temperature were also found to be alive three years afterwards. Similar survival periods were noted with the strain of Listeria from a lamb.

The Antigenic structure was not studied.

Pathogenicity

Organ suspensions from the dead voles or the pure cultures proved fatal to white mice in two-five days after intraperitoneal, intravenous or subcutaneous

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inoculation. Administration per os also set up the disease in six out of seven mice, death occurring in seven to 17 days. A typical Anton's reaction followed the instillation or gentle rubbing of the supra-orbital sac with a drop of broth culture in two guinea-pigs and one rabbit; purulent conjunctivitis was shown by the third or fifth day and the cornea also became involved. Resolution occurred by the 10th-18th and 25th days, respectively, only an opaque spot remaining on the cornea of the inoculated rabbit.

Four rabbits were inoculated intravenously with 0.5 c.c. dilutions of a 24-hour broth culture grown at about 22° C. The largest dose which was used represented approximately 31,500,000 organisms (number obtained by deep plating of serial dilutions at 37° C.); this proved fatal in 48 hours, the rabbit showing focal necrosis of the liver and spleen. The remaining rabbits, which had been inoculated with 10,584,000, 3,150,000 and 105,840 organisms, respectively, survived and showed an increase in the percentage of monocytes up to 18 per cent., 15.5 per cent. and 30 per cent., respectively, 72 hours after inoculation.

Discussion

Although *Listeria monocytogenes* was isolated from the seven voles, deaths from Listeriasis were never observed at this Institute in the other species of experimental animals. The voles were obtained from the same outside source on two separate occasions, and in the process of acclimatisation suffered losses through Listeriasis as well as other diseases. The possibility that they were carriers of the organism on purchase is likely.

The origin of outbreaks of Listeriasis in man or domestic animals is rather obscure. The disease of ruminants is reported to occur more frequently in winter and apparently more often in vermin-infested farms. The hypothesis that rats or other vermin may represent a natural reservoir of the disease has not received much support (Webb, 1943), although Macchiavello (1942) records the isolation of Listeria from the viscera of a wild rat in Brazil.

The findings recorded here, although referring to voles kept under laboratory conditions, suggest the possibility that wild voles might be a vector of Listeriasis to domestic animals. Nevertheless, the possibility of a soil origin of the infection must be considered in view of the resistance of the micro-organism, its prolonged survival at room temperature and its many similarities to *Erysipelothrix rhusiopathiæ*.

Conclusions

From two separate outbreaks in voles (Microtus agrestis) kept under laboratory conditions, strains of *Listeria monocytogenes* were isolated. They behaved like the other strains described in the literature. Four vole strains and the lamb strain remained alive at room temperature for more than three years.

The hypothesis that wild voles may be a natural source of the disease in domestic species is discussed.

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A PHARMACOLOGICAL STUDY OF THE EGYPTIAN PLANT, EUPHORBIA PEPLUS

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THE Euphorbiaceæ forms one of the most important botanical orders, containing useful medicinal plants, and the Euphorbia forms the most important genus within the order. Although Euphorbia peplus has been stated to be of importance in medicine, little is known about its medicinal properties. Because of the incomplete investigation of the Euphorbias, the present writer had proposed to undertake a thorough investigation upon one of the common species prevalent in Egypt, namely, Euphorbia peplus, the pharmacology and toxicity of which are to be discussed in the following pages. A brief description is given of this plant, as well as a summary of its chemical analysis (Sharaf, 1947) as an introduction to its pharmacology and toxicity.

In regard to its description, Euphorbia peplus is an annual plant, small glabrous, light weed, 20-30 in. high, branching near the apex. Leaves are tender, entire, petioled, obovate, 0.5 - 2.5 cm. long, obtuse or retuse. Umbel of repeatedly forked rays often occupying the greater part of the plant. Capsule is ovate, 1.5 mm. long. Seed small, ovate, oblong, with a longitudinal groove on each of the two compressed ventral sides, and on the dorsal side there are two rows of four pits each, light grey in colour, with a whitish caruncle attached to the upper end of each seed. The plant was found to flower usually at the end of November and sometimes a few weeks later, especially in cold weather or on damp soil.

In regard to its chemical investigation, it was found by the present writer that the air-dried Euphorbia peplus grown in Egypt contains 7 - 8 per cent. of resin, 0.13 per cent. of alkaloidal residue, and about 3 per cent. of glucosidal substance, as shown by several extractions.

EXPERIMENTAL

PART I

Pharmacological Actions

In the pharmacological experiments on different organs, the resin was always removed from the extract so as not to vitiate the results. These experiments may be summarised as follows:—

1. *Action on Blood Vessels*

METHOD: Trendelenberg's method was used for the perfusion of toad's blood vessels. Ringer's solution flowed from one bottle into the blood vessels, the pressure being regulated so that the flow per minute became about 20 drops. The flow of Ringer's solution was then closed, and that of the drug opened.

RESULTS: From these experiments on pithed toads it was seen that the drug produced marked dilatation of the blood vessels in 1 : 2,000 to 1 : 5,000 concentrations, while in 1 : 10,000 to 1 : 20,000 concentrations it produced little effect, and in lower concentrations it had practically no effect.

2. *Action on Toad's Heart*

METHOD: The method used was a modified Syme's method for perfusion of isolated toad's heart. The larger flask of the apparatus contained Ringer's solution, and the smaller one, the extract to be perfused in solution. When the heart was beating steadily, the clip of the smaller flask was opened and that of the larger one was shut. A sample tracing is shown in Fig. 1.

RESULTS: The drug was found to produce a definite depression on the force of contraction of the heart. With concentrations up to 1 : 2,000 there occurred marked prolonged depression, but below this concentration and up to 1 : 10,000 there was little effect, while with still lower concentrations the effect was very slight.

3. *Action on Rabbit's Head*

METHOD: The method used was a modified Gunn's in which the coronary vessels of the freshly excised rabbit's heart were perfused through the aorta. The coronary flow was recorded in each case by accurately measuring the flow per minute. The cardiac contractions were recorded by a lever connected to a hook inserted into the left ventricle. A sample tracing is shown in Fig. 2.

RESULTS: The curve recorded a depressing action on the cardiac muscle of the rabbit. The coronary flow was markedly increased, indicating definite dilatation of the coronary vessels.

4. *Action on Rabbit Intestines*

METHOD: The method was a modified Magnus method. After adjusting the apparatus, a piece of intestine, about 1 inch in length, from a freshly killed rabbit was hooked and put in the bath, and the lever was adjusted to record the movement of the intestine. A sample tracing is shown in Fig. 3.

RESULTS: There was definite relaxation of the intestinal muscle when the drug was introduced into the bath. Concentrations of 1 : 1,000 to 1 : 5,000 produced marked relaxation and depression of concentrations. This effect was gradually diminished with decreasing concentrations, and only very faint relaxation was produced by 1 : 10,000 concentration.

5. *Action on Respiration*

A. *Action on the rate*

METHOD: A special hollow rubber belt was fitted around the chest wall of an anæsthetised dog. The interior of the belt was connected by rubber tubing to a tambour, the movements of which were recorded by a lever which registered the respiratory movements on a Kymograph drum paper.

RESULTS: The alcoholic extract of the drug after being injected intravenously into the femoral vein was found to produce diminution in the rate of respiration.

B. *Action on the Bronchioles*

METHOD: The method adopted was to insert a special metal cannula in a slanting direction through the intercostal muscle into the pleural cavity of an anæsthetised dog. The cannula was then attached by rubber tubing to a tambour so as to record any variation in the lung volume. Hence, the state of the bronchioles could be expressed. Artificial respiration was performed through a

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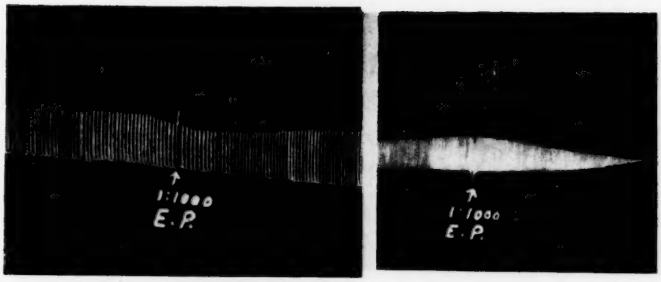

FIG. 1
Showing the effect of the extract, after removal of its resin, on toad's heart, the right tracing showing that continuous perfusion produces stoppage of the heart.

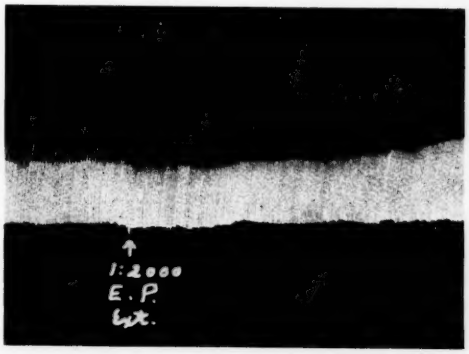

FIG. 2
Showing depressing effect of the extract, after the removal of the resin, on the rabbit's heart.

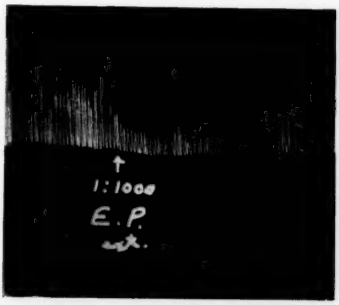

FIG. 3
Showing the relaxing effect of the extract, after removal of its resin, on rabbit's intestine.

FIG. 4
The upper tracing shows the relaxing effect of the extract, after removal of its resin, on the bronchioles in an anæsthetised dog. The lower tracing shows the concurrent effect in producing a fall in the blood pressure.

Article by A. A. Sharaf]

glass cannula inserted into the trachea, so as to allow a constant volume of air to enter the lungs. A sample tracing is shown in Fig. 4.

RESULTS: Injected into the femoral vein a solution of the drug caused an increase in the lung-volume, through dilatation of the bronchioles.

6. *Action on the Blood Pressure*

METHOD: The blood pressure in the carotid artery was recorded through a cannula containing a half-saturated solution of sodium sulphate by means of a lever on Kymographic drum paper. The drug solution was injected intravenously into the femoral vein. A sample tracing is shown in Fig. 4.

RESULTS: The drug solution lowered the blood pressure, an effect which varied according to the doses given.

PART II

Toxicity of the Plant

From toxicity experiments performed by the present writer on different animals, preparations of the plant were found to be toxic. The toxicity experiments were carried out on toads, white rats and dogs, and the plant preparations were given per os, subcutaneously or intravenously. The toxicity may be summarised as follows:—

1. *Toxicity varies according to species of animals*

(*a*) TOADS: Toads were found to be resistant. Doses as large as 31 g. of the extract per kg. body weight produced no death within 24 hours, but spasms in the hind limbs, inability to move and general depression were noticed. Therefore, it may be concluded that the drug is practically non-lethal to toads.

(*b*) WHITE RATS: They were found to be less resistant. Only large doses, however, proved fatal within 24 hours. The rats in which symptoms were produced showed apathy and dullness, staggering movements and inappetence.

(*c*) DOGS: Several dogs were given different amounts of the extract either per os in non-anæsthetised dogs or by intravenous injection in anæsthetised dogs. They were found to be more susceptible. When the extract with its resin was administered by the mouth, it did not cause death within the period of observation, but produced only symptoms of gastro-enteritis, such as nausea, vomiting and diarrhœa. Death did not occur, it seems, because the drug was partially always vomited. When given by intravenous injection to anæsthetised dogs, the extract, after the removal of its resin, was noticed to be highly toxic and death was always the result after shivering and irregularity in respiration and pulse.

2. *M.L.D. of the extract*

(*a*) IN TOADS: Different amounts of the alcoholic extract, after removing its resin, were injected into the ventral lymph sac in different groups of toads. The M.L.D. was probably very high, inasmuch as doses as large as 31 g. of extract per kg. body weight did not cause death.

(*b*) IN RATS: Different groups of white rats were injected subcutaneously with various amounts of the extract after removing its resin. The M.L.D. of the extract given by this way was found to be 11 g. per kg. body weight.

(*c*) IN DOGS: The M.L.D. was found to be about 0.275 g. of the extract per kg. body weight when given by intravenous injection (after the removal of

its resin) to anæsthetised dogs, but in non-anæsthetised dogs when the drug containing its resin was given per os, death did not occur even with large doses.

3. *Toxic principles and their effects*

(*a*) THE RESIN, amounting to about 7½ per cent. of the powdered plant, was experimentally found to produce in dogs symptoms of gastro-enteritis, such as nausea, vomiting and purgation.

(*b*) THE ALKALOID RESIDUE, amounting to about 0.13 per cent. of the powdered plant, was experimentally found to be the cause of a series of systemic effects, namely, of dilating the blood vessels, lowering the blood pressure, depressing the cardiac muscle, dilating the bronchial and intestinal muscles, etc. The toxic symptoms are characterised by dullness, depression, staggering if moved and off food.

(*c*) THE GLUCOSIDAL SUBSTANCE, amounting to about 3 per cent. of the powdered plant, was found to produce systemic effects resembling more or less those of the alkaloid.

4. *Post-mortem findings*

(*a*) The most characteristic were those of highly congested lungs and congestion of other internal organs, indicating that the drug in fatal doses has a toxic effect on the respiratory organs, and this may be considered as the main cause of death.

(*b*) The heart was highly congested and dilated, indicating that, besides the respiratory failure, the drug has a depressant effect on the heart, which may be considered as a secondary cause of death. The point was verified when a fatal dose of the extract was given to anæsthetised dogs; it stopped respiration first, and when the sheet was opened, the heart was found to continue beating for about five minutes after cessation of respiration. These findings were produced from the extract after the removal of its resin.

5. *Cause of death*

(*a*) RESPIRATORY FAILURE: Respiration was noticed to be irregular, then stopped before the heart, in anæsthetised dogs. The post-mortem appearances indicate this to be the main cause of death.

(*b*) CIRCULATORY FAILURE: In anæsthetised dogs the blood pressure was found to be irregular, rising and falling, then stopped after the respiration. The post-mortem appearances and the stoppage of the heart after respiration denotes that the circulatory failure is a secondary cause of death.

Discussion

1. With regard to the action of the different species of Euphorbia, little has been written concerning the action of Euphorbia peplus in particular. The statement of Martindale and Westcott, Gehes and the British Pharmaceutical Codex that Euphorbia peplus is effective in suppressing attacks of asthma is verified by the writer, who has found, moreover, that it has sufficient relaxing effects on the bronchioles to be an asthma remedy. It has been stated by Greenish, Youngken, R. C. Warn, R. N. Chopra, and others, that Euphorbia pilulifera also has been used with success in asthma, bronchitis and other respiratory diseases.

XUM

Moreover, the writer has proved that an extract of this plant after the removal of its resin produces: (1) Dilatation of the blood vessels; (2) depression of the cardiac muscle; (3) coronary dilatation; (4) relaxation of the intestinal muscle; (5) dilatation and relaxation of the bronchioles; and (6) a lowering effect on the blood pressure.

2. As regards toxic action, it is stated by H. C. Wood and by Gunn that Euphorbia pilulifera has a direct depressant action on the heart and respiration. Moreover, Gunn states that in large doses it paralyses the heart and respiration. This statement, in connection with Euphorbia peplus, is also true, in so far as it was proved experimentally by the present writer that Euphorbia peplus is a direct depressant to the cardiac muscle and produces death, which is due to both respiratory and circulatory failure. Moreover, the writer proved experimentally that depression of the heart beat was due to direct action on the cardiac muscle, as this depression took place even after the ganglia and nerve endings had been paralysed, namely, by action of nicotine and atropine respectively.

The statement of H. G. Long and others that species of Euphorbia afford products which act powerfully as emetics and cathartics, and in overdoses produce serious results, has been experimentally confirmed by the writer, who has shown, moreover, that Euphorbia peplus possesses the above-mentioned toxic effects in virtue of its resin content. Moreover, the writer concludes that the toxicity of the plant differs in the different species of animals: toads were resistant to doses high as 31 g. per kg. body weight of the extract, while rats are somewhat resistant to doses as high as 11 g. of the extract per kg. body weight. Dogs were found to be still more susceptible, the M.L.D. being estimated to be 0.275 g. per kg. body weight when given intravenously to anæsthetised dogs.

3. The low toxicity, especially when administered per os, of the plant or its extract, and the pharmacological characteristics of its several active principles, suggest therapeutic possibilities. The resin has been proved experimentally to be a safe purgative for dogs (Sharaf, 1947), and it may also prove to be a useful purgative for other animals. The use of the extract, after removal of its resin, is strongly suggested in asthma and bronchiectasis, as it has been verified experimentally to produce dilatation of the bronchioles. As a remedy for asthma in this manner, the extract is still being investigated experimentally by the present writer, who has obtained favourable results in animals and some human patients in this connection. The extract has also a definite lowering effect on the blood pressure, as it has been proved to produce direct dilatation of the blood vessels; hence, its use is suggested in arterial hypertension. The extract, moreover, can be given in all conditions associated with spasms such as renal colic. It may be concluded that Euphorbia peplus extract resembles nitrates in its therapeutic action in this respect as an antispasmodic.

Conclusions

1. The air-dried Euphorbia peplus grown in Egypt was found to contain about 7.5 per cent. of resins, 0.13 per cent. of alkaloidal residue, and 3 per cent. of glucosidal substance.

2 (*a*) The resin was experimentally found to produce symptoms of gastro-enteritis in dogs, such as nausea, vomiting and purgation.

(*b*) The alkaloidal residue was found to produce dilatation of the blood vessels, lowering of the blood pressure, dilatation of the bronchial and intestinal muscles, etc.

(*c*) The glucosidal substance was found also to produce systemic effects resembling more or less those of the alkaloidal residue.

3. The plant was proved to be toxic and the toxic range differs in different animals: toads were found to be resistant, rats less resistant, dogs more susceptible, and the herbivora not tested.

4. The therapeutic possibilities, in view of the proved low toxicity and the pharmacological characteristics, can be summarised as follows:—

(*a*) The resin has been proved to be safe for use as a purgative for dogs when given as coated pills.

(*b*) The extract, after removal of its resin, can be used therapeutically as a remedy for asthma, bronchiectasis and similar conditions.

(*c*) It may be used in conditions associated with arterial hypertension.

(*d*) It can be used also as antispasmodic in spasmodic conditions.

Acknowledgment

I should like to express my thanks to Dr. M. Imara, Professor of Toxicology; to Dr. I. R. Fahmy, Professor of Pharmacognosy; and to Dr. M. A. Sherif, Professor of Pharmacology, Faculty of Medicine, Fouad 1st University, for their valuable help.

I owe grateful acknowledgment to J. T. Edwards, Visiting Professor and Temporary Dean of School of Veterinary Medicine, Fouad 1st University, for his help in writing this paper.

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REVIEW

Veterinary Protozoology. By ULICK F. RICHARDSON, B.Sc., M.R.C.V.S. Published Edinburgh by Oliver and Boyd. Price: 18s. net.

BY producing this book the author succeeds in filling a gap which has existed all too long in the veterinary literature of this country. Until the publication of this volume, there has been no comprehensive textbook dealing solely with pathogenic Protozoa of veterinary importance, and the veterinarian has had to rely on the small sections dealing with protozoology which are included in textbooks of pathology or of general parasitology, or on books devoted to medical and veterinary protozoology, where the emphasis has always been on the medical aspect. The information contained in these works is often incomplete and inadequate in respect of the vast amount of recent knowledge which has accumulated, particularly with regard to the parasitic Protozoa of the tropics and the modern clinical methods of treating protozoon-induced diseases. Detailed information on many of the important parasitic Protozoa of domestic animals is not readily available to the veterinary surgeon, dispersed as it is in the form of original papers throughout the world's medical, veterinary and agricultural journals. The author is to be complimented on producing a book in which nearly all of the most recent information on the pathogenic Protozoa of domestic animals has been brought together, and for the painstaking effort which he has obviously made in order to make the book as comprehensive as possible. The book includes descriptions of the protozoon parasites of domestic mammals and birds, with their morphological characters and distinguishing features, their developmental cycles and methods of transmission, and describes the various measures which are employed to prevent their spread. The author also deals with the nature of the diseases produced by the various types of Protozoa, with their clinical symptoms and pathology, and describes in considerable detail various methods of treatment by chemotherapy or by immunisation. The book is divided into sections, each dealing in detail with the various parasites of veterinary importance in the four main classes of Protozoa, with an additional chapter on unclassified organisms, like Sarcocystis, Toxoplasma and Rickettsia. At the end of each chapter the author gives a useful list of references of original papers dealing with the main points in the text, but on occasion he quotes a research worker of whose paper no mention is made in the list of references. Each protozoon species is dealt with in considerable detail, with a description of the symptoms of the disease produced, methods used in diagnosis of the condition, and the most recent methods of treatment and control. Included in the book there is a chapter on chemotherapy, in which is given the chemistry and action of the various substances which have been shown to be active against Protozoa. There is also a chapter on technique, of interest particularly to workers in the tropics, in which the author describes the main staining methods used for Protozoa, the methods employed in examining fæces, smears, etc., for the presence of the parasites, methods employed in culturing Protozoa, and details of the serological tests for some of the parasitic Flagellates. The sections dealing with the general morphology of Protozoa are somewhat scanty and inadequate and are not up

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to the standard of the rest of the book, and at times the classification tends to be confusing. The book, generally, gives the impression that the author presupposes the reader to have a fair knowledge of zoology: for example, he seldom refers to the vector of a parasite by its common name, but uses zoological nomenclature, which would necessitate a student using a textbook of entomology in conjunction with the text. From the point of view of the student, a schematic diagram of the life cycle of each of the major types of protozoon parasite would have helped him to follow the detailed description in the text. Some of the diagrams, e.g., figures 1 and 2, have no legend and are not referred to in the text. It is hoped that the book, when it goes into its second edition, which certainly it must do, will have some of its faults remedied. The book will provide the student with a vast amount of information on the protozoon parasites of veterinary importance, will be of great value to the practitioner in the diagnosis and treatment of protozoon diseases, and will appeal particularly to veterinary surgeons working in the tropics.

J.H.J.

NEWS

PENICILLIN VETERINARY CERATE.—On August 9, Glaxo Laboratories, Ltd., of Greenford, Middlesex, introduced Penicillin Veterinary Cerate Glaxo for the treatment of bovine mastitis. The cerate is supplied in boxes containing 16 tubes, sufficient for a four-day course of treatment for one cow. Each tube contains a single dose of 100,000 international units of penicillin. The penicillin is presented in a non-irritant base of arachis oil with 4.5 per cent. white beeswax to secure a steady and prolonged action in the udder.

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